Anti-diabetic Activity of Aqueous Extract of *Chrysophyllum albidum* Stem Bark, its Fractions and Toxicity in Alloxan-induced Diabetic Rats

BY

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A THESIS SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF ILORIN, ILORIN, NIGERIA, IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY (Ph.D.) DEGREE IN BIOCHEMISTRY

JUNE, 2018

DECLARATION

I, Babalola Ola YUSUF (Matric Number – 02/55EH203) declare that this thesis entitled "Anti-diabetic activity of aqueous extract of *Chrysophyllum albidum* stem bark, its fractions and toxicity in alloxan-induced diabetic rats*"* is the result of a study carried out by me under the supervision of Professors M.A. Akanji and M.T. Yakubu in the Department of Biochemistry, University of Ilorin, Nigeria. I confirm that this thesis presents the results of my findings and that its contents are entirely my ideas unless otherwise stated with appropriate referencing. This thesis has not been submitted to any University nor is it before any other University for consideration for the award of a Doctor of Philosophy (Ph.D.) Degree in Biochemistry.

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CERTIFICATION

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DEDICATION

This thesis is dedicated to my father, Alhaji Dauda Yusuf Atesinse Al-Imam for the risk he took in enrolling me in school at the time no one in the family dare go to school.

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ABBREVIATIONS

ADA	American Diabetes Association
AI	Atherogenic Index
ALP	Alkaline Phosphate
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
DKA	Diabetic Ketoacidosis
DNS	3,5-dinitrosalicyclic acid
DTNB	5,5'-dithio-bis(2-nitrobenzoic acid)
G6PDH	Glucose-6-Phosphate Dehydrogenase
H&E	Haematoxylin and Eosin
HDLC	High Density Lipoprotein Cholesterol
HHS	Hyperglycaemic Hyperosmolar State
HPLC	High Performance Liquid Chromatography
IDDM	Insulin Dependent Diabetes Mellitus
IFG	Impaired Fasting Glycaemia
IGT	Impared Glucose Tolerance
LA	Lactic Acidosis
LDLC	Low Density Lipoprotein Cholesterol
MDA	Malondialdehyde
NIDDM	Non-Insulin Dependent Diabetes Mellitus
OGTT	Oral Glucose Tolerance Test
PCV	Packed Cell Volume
pNPG	*p*-nitrophenyl-α-D-glucopyranoside
PNPP	*P*-nitrophenyl Phosphate

RBC	Red Blood Cell
SOD	Superoxide Dismutase
TAG	Triacylglycerol
TBA	Thiobarbituric Acid
TLC	Thin Layer Chromatography
VLDLC	Very Low Density Lipoprotein Cholesterol
WHO	World Health Organization

ABSTRACT

Diabetes mellitus is a chronic metabolic disease that is caused by a relative lack of insulin and/or reduced insulin activity resulting in hyperglycaemia. The rise in poverty level coupled with side effects of available anti-diabetic drugs necessitated the evaluation of a medicinal plant, *Chrysophyllum albidum*, claimed to be used in the management of diabetes. The objectives of the study were to: (i) evaluate the anti-diabetic activity of aqueous extract of *C. albidum* stem bark (AECASB); (ii) carry out solvent partitioning of AECASB and evaluate their anti-diabetic activity; (iii) carry out bioactivity-guided chromatographic fractionation (CF) of the most effective solvent-partitioned fraction (SPF) of AECASB and evaluate their anti-diabetic activity; (iv) identify the bioactive principles in the most effective CF of AECASB; (v) carry out safety evaluation of the antidiabetic principle(s)-rich CF of AECASB and (vi) propose the mechanism of action of the antidiabetic principle(s)-rich CF of AECASB.

A total of 340 female rats (180.80 ± 8.50g) were assigned into group A (received 1.0 ml of distilled water) while the diabetic rats {induced by intraperitoneal administration of 120 mg/kg body weight (bw) of alloxan} in groups B, C, D, E, F, G were orally administered distilled water, glibenclamide (reference antidiabetic drug), 25, 50, 100 and 200 mg/kg bw of AECASB respectively, once daily for 14 days. Crude, SPFs (ethyl acetate, residue, n-butanol) and CF (1-10) were also screened. Biochemical assessments and histological examinations were also carried out. Data were analysed with Analysis of Variance and Tukey's *post-hoc* test at $p < 0.05$.

The findings in this study revealed that:

i. AECASB reversed the alloxan treatment related increases in fasting blood glucose (FBG), serum urea, creatinine, hepatic glucose (HG), amylase activity and related decreases in

albumin, glycogen, erythrocytic indices, glucokinase (GK) and glucokinase-6-phosphate dehydrogenase (G6PDH) activities.

ii. AECASB also restored the deranged histoarchitecture of the pancreas caused by alloxan;

iii. ethyl acetate-partitioned fraction reduced ($p<0.05$) FBG, HG, α-amylase activity, total cholesterol, triglycerides, malondialdehyde (MDA), ameliorated the obliteration observed in pancreas due to alloxan treatment while glycogen, high density lipoprotein (HDL), G6PDH, SOD and catalase activities increased significantly ($p<0.05$);

iv. Chromatographic fractions F5 and F7 decreased ($p<0.05$) FBG, HG, organ body-weight ratio, glycosylated haemoglobin, C-reactive protein, glucose-6-phosphatase and fructose-1,6-bisphosphatase activities, TC, triglycerides, atherogenic index, MDA while BW, serum insulin, glycogen, HDL, GK, G6PDH, catalase, SOD activities significantly ($p<0.05$) increased;

v. procyanidin, stigmasterol, catechin, epicatechin, epigallocatechin, hexadecane, eleagnine, octadecanoic acid and eicosane were detected in F7;

vi. CFs 5 and 7 did not alter urea, creatinine, albumin, bilirubin, alkaline phosphatase, alanine aminotransferase and aspartate aminotransferase activities;

vii. catechin, stigmasterol and procyanidin B5 are the likely bioactive principles in CF7; and

viii. mode of anti-diabetic action of AECASB and F7 may be via regenerating the pancreas and enhancing the activities of glucose metabolising and antioxidant enzymes.

The study concluded that CF7 produced the most profound anti-diabetic activity and is relatively safe. AECASB can be explored as lead drug for the management of diabetes.

CHAPTER ONE

INTRODUCTION

1.1 Background of Study

Diabetes mellitus is a protracted metabolic ailment that is caused by a relative lack of insulin and/or reduced insulin activity that result in hyperglycaemia (Harris *et al*, 1998; Kamtchouing *et al*, 2006 and Ponugoti *et al*, 2013). According to WHO (2016), diabetes is categorized into three major types viz; Type 1 diabetes (formerly known as insulin-dependent, juvenile or childhood-onset diabetes) which is branded by undersupplied insulin production in the body; type 2 diabetes (formerly called non-insulin-dependent or adult-onset diabetes) results from the body's ineffectual use of insulin; and gestational diabetes which is a temporary condition that ensues in pregnancy and carries long-standing risk of type 2 diabetes (Finucane *et al.,* 2014). A number of lifestyle factors influencec the development of diabetes and these include obesity, age, dearth of physical activity, poor diet, stress, and urbanization (Bi *et al.,* 2017). Signs and symptoms include polydipsia, polyurea, skin dryness, rapid weight loss, polyphagia, nausea, Irritability, hazy vision, skin infections and wounds that heal slowly (Ramachandran, 2014; Lal, 2016).

Impediments such as nephropathy, neuropathy, retinopathy, hepatomegaly, diabetic coma or ketoacidosis, high blood pressure, kidney disease, stroke, amputation, hypoglycaemia, stillbirth and perinatal death (in case of gestational diabetes) may ensue if diabetes is not pickled (Tepe *et al*., 2007; Lal, 2016).

A number of management options including dietary and exercise modifications and pharmacological medications (such as insulin, metformin and glybenclamide) have been engaged in the management of diabetes. Some side effects such as abdominal discomfort, anorexia, bloating, diarrhoea and lactic acidosis (Ikeda *et al.,* 2000) are associated with pharmacological medications. Safety concern has therefore become a factor militating against

the use of conventional medicines (WHO, 2005). To this upshot, a number of herbs are broadly used as foods, dietary supplements and drugs (Santini and Novellino, 2014; Nasri *et al.,* 2015) and have either been scientifically proven or rely largely on the history and ethnopharmacological prospects (Lapenna *et al.,* 2015; Mocan *et al.,* 2016). One of such plants is *Chrysophyllum albidum*

Chrysophyllum albidum stem bark has been used to manage diabetes by some locals in Ifelodun Local Government Area of Kwara State without scientific evidence to either validate or discourage its usage. The isolation and identification of bioactive principle(s) of *C. albidum* stem bark as well as its toxicity has also not been reported. This study therefore, was designed to provide information on antidiabetic principle(s) of aqueous extract of *Chrysophyllum albidum* stem bark and its toxicity in alloxan-induced diabetic rats.

1.2 Statement of the Problem

Effective management of diabetes is still a defy despite tireless efforts of investigators across the globe to abate the menace. Though numerous oral antihyperglycemic agents are available for clinical use, they are not without the characteristic side effects (Kameswara *et al.,* 1997; Holman and Turner, 1991). The increase in poverty levels, poor availability of medical facilities and drug safety considerations has become factors militating against the use of conventional medicines (WHO, 2005). Therefore, there is a growing need to develop substitutes from natural sources that is safe, cheap, readily available and affordable to the masses.

1.3 Justification for the Study

Despite the wide use of various parts of *Chrysophyllum albidum* by traditional medicine practitioners in managing diseases including diabetes (Houessou *et al.* 2013), there is no evidence based research on anti-diabetic effects of the stem bark, its bioactive

principles, its mode of action as anti-diabetic agent, as well as safety, hence the need for this study.

1.4 Objective of the Study

The overall objective of this study was to confirm or refute the acclaimed antidiabetic potency of aqueous extract of *Chrysophyllum albidum* stem bark and provide information on its mode of action and toxicity as antidiabetic agent.

The specific objectives of the study included the following:

i. evaluation of the anti-diabetic activity of aqueous extract of *C. albidum* stem bark;
ii. carrying out solvent partitioning of the aqueous extract of *C. albidum* stem bark using organic solvents of various polarities;
iii. determining *in vitro* anti-diabetic and antioxidant activity of the aqueous and solvent-partitioned fractions of *C. albidum* stem bark;
iv. carrying out dose-dependent anti-diabetic study of the most pharmacologically active solvent-partitioned fraction of *C. albidum* stem bark;
v. conducting the bioactivity-guided fractionation of the most pharmacologically active solvent- partitioned fraction of *C. albidum* stem bark in alloxan-induced diabetic rats;
vi. identifying the bioactive principles in the most effective fraction of *C. albidum* stem bark using HPLC analysis;
vii. evaluating the safety of the antidiabetic principle(s) – rich fraction of *C. albidum* stem bark using biochemical parameters of liver and kidney function;
viii. proposing the possible mechanism of action of the antidiabetic principle(s) – rich fraction of *C. albidum* stem bark.

1.5 Experimental Design

The methodology employed in this study were designed as depicted in Figure 1

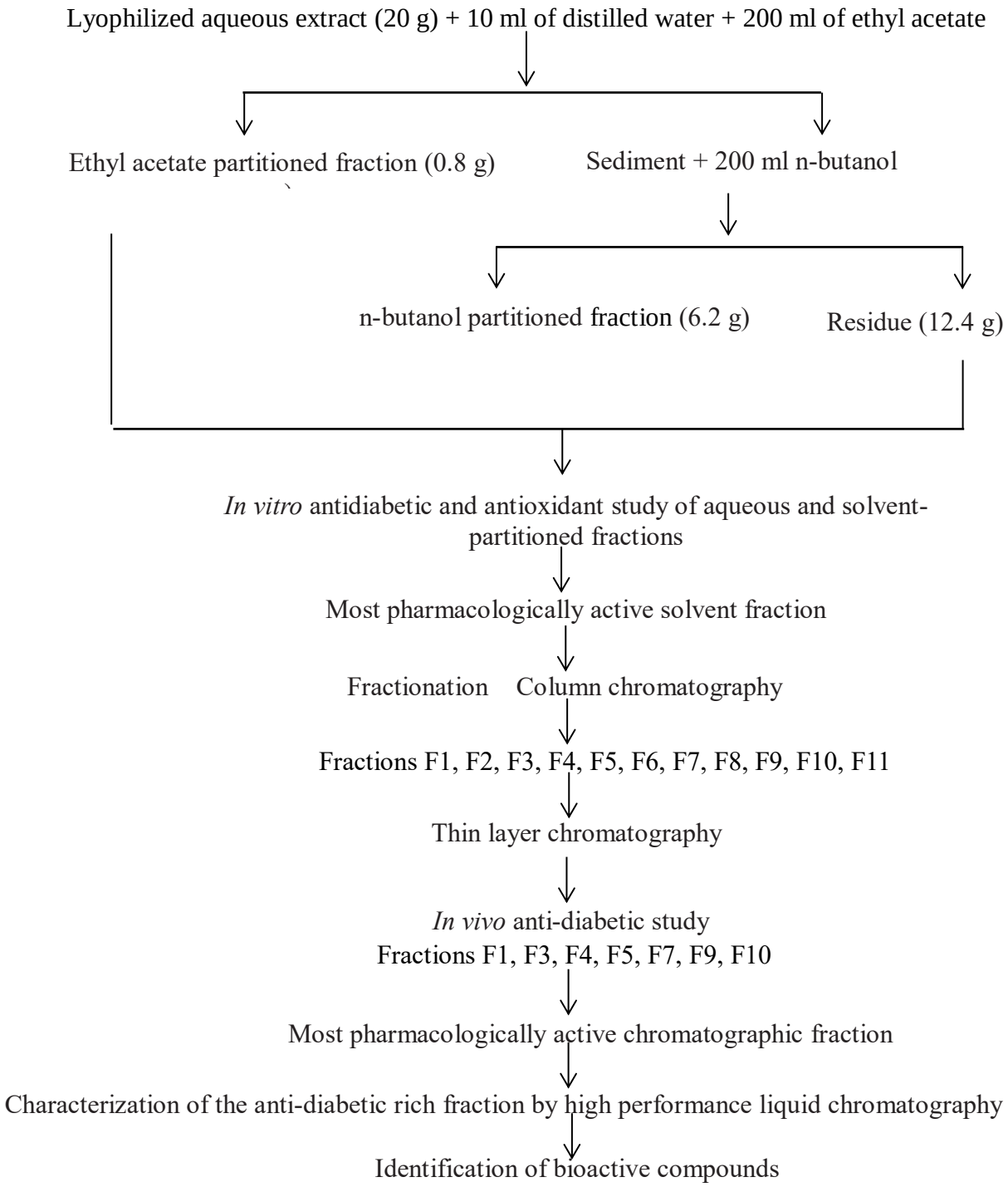


Figure 1: Bioactivity-guided isolation of anti-diabetic principles from *C. albidum* stem bark

1.6 Significance of the Study

The significance of the study is to identify the bioactive agent(s) with a view to formulating a cheaper and safer drug lead for the management of diabetes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes

The term ‘diabetes’ is derived from the Greek word meaning siphon or marked loss of water by urination, or polyuria. The word *mellitus* comes from the Latin word for *sweet* or *honey*. This differentiates diabetes mellitus (sweet urine disease) from diabetes insipidus (bland urine disease), a disease associated with the posterior pituitary gland (Kobberling, 1971).

2.1.1 Diabetes Mellitus

Diabetes mellitus (a pancreatic disorder) is a chronic metabolic disease caused by an absolute or relative lack of insulin and/or reduced insulin activity, which results in hyperglycaemia and abnormalities in carbohydrate, protein, and lipid metabolism (Harris *et al.,* 1998; Kamtchouing *et al*, 2006 and Ponugoti *et al*, 2013).

Obesity, poor diet and sedentary life style which are major components of the metabolic syndrome, have been linked to the principal causative factors for the prevalence of this disease (Huang *et al.,* 2009; Sperling *et al.,* 2015; Waltenberger *et al.*, 2016). In addition to hyperglycemia, other factors such as hyperlipidemia and oxidative stress play great role in pathogenesis of diabetes leading to high risk of complications (Kangralkar *et al*., 2010).

2.2 Symptoms of Diabetes

Clinical symptoms of diabetes include polydipsia (excessive thirst), polyuria (excessive urination), polyphagia (hunger), rapid weight loss recurrent infections, feeling of extreme weakness and fatigue, eye symptoms, erectile dysfunction, sores that heal slowly, loss of consciousness (ADA, 2009b).

2.3 Types of Diabetes Mellitus

Various types of diabetes are known but the classification by WHO (2016) that is generally adopted include the following:

2.3.1 Type 1 diabetes Mellitus

Type 1 diabetes mellitus (previously known as insulin-dependent, juvenile or childhood-onset diabetes) is characterized by deficient insulin production in the body. It affects over 40 million people worldwide (WHO, 2016) and is most frequently diagnosed between the ages of four and five years or in adolescence (Ozougwu *et al.*, 2013). It is an autoimmune disease that results from the specific destruction of insulin-producing β-cells in the pancreas (Atkinson *et al.,* 2014). Apart from its strong genetic basis, there has been a progressive rise in the incidence of type 1 diabetes in recent decades, indicative of a strong increasing contribution from the environment. Possible environmental influences include hygiene (Vatanen *et al.,* 2016), antibiotic use (Livanos *et al.,* 2016) and diet (Thorburn *et al.,* 2014). People with type 1 diabetes require insulin administration to regulate the amount of glucose in their blood (Rother, 2007; Rajashree *et al.,* 2012; Tumminia *et al.,* 2015). Symptoms include excessive urination and thirst, constant hunger, weight loss, vision changes and fatigue (Rother, 2007).

2.3.2 Type 2 Diabetes Mellitus

Type 2 diabetes (formerly called non-insulin-dependent or adult-onset diabetes) results from the body's ineffective use of insulin. Type 2 diabetes accounts for about 95% of people with diabetes around the world (Fradkin, 2012). This type is due primarily to lifestyle factors and genetics (Risérus *et al,* 2009). A number of lifestyle factors including obesity, lack of physical activity, poor diet, stress, and urbanization are known to be important to the development of type 2 diabetes (Malik *et al.*, 2013). Symptoms may be similar to those of type 1 diabetes, but are often less marked or absent. As a result, the disease may go

undiagnosed for several years, until complications have already arisen. For many years, type 2 diabetes was seen only in adults but it has begun to occur in children (ADA, 2017).

2.3.3 Impaired Glucose Tolerance and Impaired Fasting Glycaemia

These are intermediate conditions in the transition between normal blood glucose levels and diabetes (especially type 2), and it is refers to as prediabetes. These conditions occour when the fasting plasma glucose level ranges between 100-140 mg/dL (5.6- 6.9 mmol/L) or 140-199 mg/dL (7.8-11.0 mmol/L) in 2 hours 75 g OGTT test or A1C of 5.7-6.4% (ADA, 2017). People with Impaired glucose tolerance (IGT) or impaired fasting glycaemia are at increased risk of heart attacks and strokes both of which can be delayed or prevented by metabolic control (ADA, 2014).

2.3.4 Gestational Diabetes Mellitus (GDM)

This occurs in pregnancy and carries long-term risk of type 2 diabetes (Finucane *et al.,* 2014). Gestational diabetes is diagnosed through prenatal screening, rather than reported symptoms. The condition may improve or disappear after delivery (ADA, 2014). It occurs when fasting blood glucose values equals or exceed 92 mg/dL (5.1 mmol/L) or 180 (10.0 mmol/L) and 153 mg/dL (8.5 mmol/L) for 1 and 2 hours OGTT respectively at 24-28 weeks of gestation in women not previously diagnosed with overt diabetes (ADA, 2017). Women with gestational diabetes are at amplified danger of some impediments during pregnancy and delivery, as are their babies (ADA, 2018).

2.4 Diagnosis of Diabetes Mellitus

A patient is diagnosed to be diabetic when he/she has:

i. fasting plasma glucose level > 7.0 mmol/l (126 mg/dl)
ii. plasma glucose > 11.1 mmol/l (200 mg/dl) two hours after a 75 g oral glucose load as is the case of glucose tolerance test
iii. symptoms of hyperglycemia and casual plasma glucose > 11.1 mmol/L (200 mg/dl)

iv. glycated hemoglobin (Hb A1C) > 6.5% (WHO, 1999; ADA, 2009a).

2.5 Prevalence of Diabetes Mellitus

The world is experiencing increasing prevalence of diabetes (Table 1) with developing countries being majorly affected due to changes in diet, with higher fat intake, sedentary lifestyle changes, and decreased physical activity (Gupta and Kumar, 2008; Bhavsar *et al.,* 2010; Williams *et al.*, 2014).

2.6 Complications of Diabetes Mellitus

Diabetes is a sort of disorder in which the patients are at all the time on risk of complications (Wallace, 2004). Hyperglycemia, the hallmark of diabetes, contributes to the development of vascular complications through mechanisms such as activation of the polyol and hexosamine pathways, activation of protein kinase C, increased oxidative stress, increased production of advanced glycation end-products, increased synthesis of growth factors, cytokines and angiotensin II. These can, in turn, induce a diffuse endothelial dysfunction and contribute to the progressive development of micro- and macrovascular complications and multi-organ damage. Increase oxidative stress, induced by several hyperglycemia-activated pathways, is a key factor in the pathogenesis of endothelial dysfunction and vascular disease as increase production of oxidants is often associated with reduced antioxidant defenses (Chiarelli and Marcovecchio, 2013)

Complications may be macrovascular (coronary heart disease, peripheral vascular disease and stroke), microvascular (neuropathy, retinopathy and nephropathy) and both micro- and macrovascular (diabetic foot ulcer). The mortality and morbidity of diabetes are associated more with macrovascular degeneration as compared to the risks of microvascular complications (Wallace, 2004). In general, complications of diabetes mellitus can be categorized into two (Table 2) (Mohan, 2002; Wallace, 2004). In addition to the traditional

Table 1: Prevalence of Diabetes

IDF Region	people with diabetes (Millions)	
	2017	2045
Africa	156% (16)	400% (41)
South and Central America	62% (26)	100% (42)
North America and Caribbean	35% (46)	47% (62)
Middle East and North Africa	110% (39)	231% (82)
Europe	16% (58)	18.5% (67)
South-East Asia	84% (82)	155% (151)
Western Pacific	15% (159)	17% (183)
Total	425	629

Source: IDF (2017).

Table 2: Complications of Diabetes

Acute Complication	Chronic Complication
Diabetic ketoacidosis	Retinopathy
Hyperglycaemia hyperosmolar state	Neuropathy
Lactic acidosis	Renal disease
Hypoglycaemia	Heart disease and hypertension
Polydipsia and polyuria	Atherosclerosis

Source: Asmat *et al* (2015)

complications mentioned earlier, diabetes has been associated with increased rates of specific cancers, and increased rates of physical and cognitive disability (Reid *et al.*, 2012).

2.6.1 Diabetic Ketoacidosis

Diabetic ketoacidosis (DKA) is a corporate and deadly complication of type 1 diabetes, especially at the time of identification. It is defined as a triad of: hyperglycemia of plasma glucose >250 mg/dL (>13.88 mmol/L); venous pH < 7.3 and/or bicarbonate < 15 mmol/L and moderate or large ketone levels in urine or blood (Dunger *et al.*, 2004; Kitabchi *et al.*, 2006; Wolfsdorf *et al.*, 2014). It is caused by very low levels of effective circulating insulin and affiliated increase in counter regulatory hormone levels, such as glucagon, catecholamines, cortisol, and growth hormone. This combination leads to catabolic changes in the metabolism of carbohydrates, fat, and protein. Impaired glucose use and increased glucose release by the liver and kidneys result in hyperglycemia. Lipolysis leads to increased production of ketones, especially beta-hydroxybutyrate (β-OHB), ketonemia, and metabolic acidosis, which is exaggerated by ongoing fluid and electrolyte losses. In type 2 diabetes patients, DKA occurs during concomitant acute illness or during transition to insulin dependency (Rewers, 2016).

2.6.2 Hyperglycemic Hyperosmolar State

Hyperglycemic hyperosmolar state (HHS) is one of the derangements occourring in patients with diabetes mellitus (Pasquel and Umpierrez, 2014). It is an extreme elevation in blood glucose >600 mg/dL (>33.30 mmol/L) and serum osmolality >320 mOsm/kg in the absence of significant ketosis and acidosis and small amounts of ketones may be present in blood and urine (Kitabchi and Nyenwe, 2006). HHS accounts for <1% of all admissions related to diabetes but may affect up to 4% of new type 2 diabetes patients (ADA, 2001; Bradford *et al.*, 2017). It occurs at any age, but it is more predominant in elderly patients. Conditions, such as infection, cardiovascular disease and cancer, seems to be liable for the

higher mortality linked with HHS compared to DKA (Kitabchi *et al.,* 2006). The bulk of HHS episodes are occasioned by an infectious process; other factors include cerebrovascular accident, alcohol abuse, pancreatitis, myocardial infarction, trauma, and drugs (Bhansali and Sukumar, 2016). Medications affecting carbohydrate use, such as corticosteroids, thiazides, and sympathomimetic agents (e.g., dobutamine and terbutaline), may also precipitate the development of HHS (Wachtel *et al.,* 1987).

The three major biochemical events that lead to HHS are the ineffectual action of insulin, elevated levels of counter regulatory hormones (glucagon, catecholamines, cortisol, growth hormone) which result in increased hepatic glucose production and reduced glucose utilization in peripheral tissues, and dehydration and electrolyte aberrations mainly due to osmotic diuresis caused by glycosuria (Maletkovic and Drexler, 2013).

2.6.3 Lactic Acidosis

Lactic acidosis (LA) is the rise of lactic acid above 5.0 mEq/L with acidosis (pH <7.3) but without ketoacidosis. There may be low levels of ketones present (1:4 on serum dilution or β-OHB >0.4–< 0.6 mmol/L). The usual precipitating factors for LA are conditions of impaired oxygenation, such as hypoxemia, shock, sepsis, carbon monoxide poisoning, and some medications like phenformin and metformin, particularly when used by patients with renal failure. Phenformin, a biguanide, increases the risk of life-threatening LA. Also, metformin is thought to increase the risk of LA in patients with diabetes and is contraindicated in conditions associated with LA, such as cardiovascular, renal, hepatic, and pulmonary diseases, and advanced age (van Berlo-van *et al.,* 2011).

2.6.4 Hypoglycaemia

This is defined as episodes of an aberrantly low plasma glucose concentration that expose the individual to prospective damage (Ly *et al.,* 2014). It is the predominant, life-threatening grave barrier of diabetes management. It is characterized by various risk factors

and intricate pathophysiology like reduced sympathoadrenal and abridged epinephrine responses which could lead to slight cognitive injury, coma, seizure, and sudden death (Cryer, 2006). Other complications include retinopathy, neuropathy, renal disease, stroke, heart disease and hypertension among others.

2.7 Management options of Diabetes Mellitus

The aims of controlling glycaemia are to avoid acute osmotic symptoms of hyperglycemia, to avoid instability in blood glucose over time, and to prevent or delay the development of diabetes complications without adversely affecting quality of life (Rao *et al.*, 2011). This can be achieved through: dietary and exercise modifications, pharmacological agents such as insulin, oral antihyperglycaemic agents (Fowler, 2010; Ismail-Beigi, 2012) and phytotherapy (Firdous, 2014).

2.7.1 Dietary and Exercise Modifications

Medical nutrition therapy is one of the cornerstones of diabetes management. Nutrition and physical activity are important parts of a healthy lifestyle of humans, though, optimal glycemic control in diabetic patients may be achieved through dietary and exercise modifications (Stephenson *et al.*, 2014; ADA, 2017). The modification is targeted at the quantities, proportions, variety, or combinations of different foods and beverages in diets, and the frequency with which they are consumed (USDA, 2016). However, an individual consumption preference is tailored to achieve metabolic targets as there is no single choice of eating pattern (Evert *et al.*, 2013; Mathe *et al.*, 2016; Osonoi *et al.*, 2016; Shi *et al.*, 2016; ADA, 2017). This pattern is best explained using food pyramids (Figure 2).

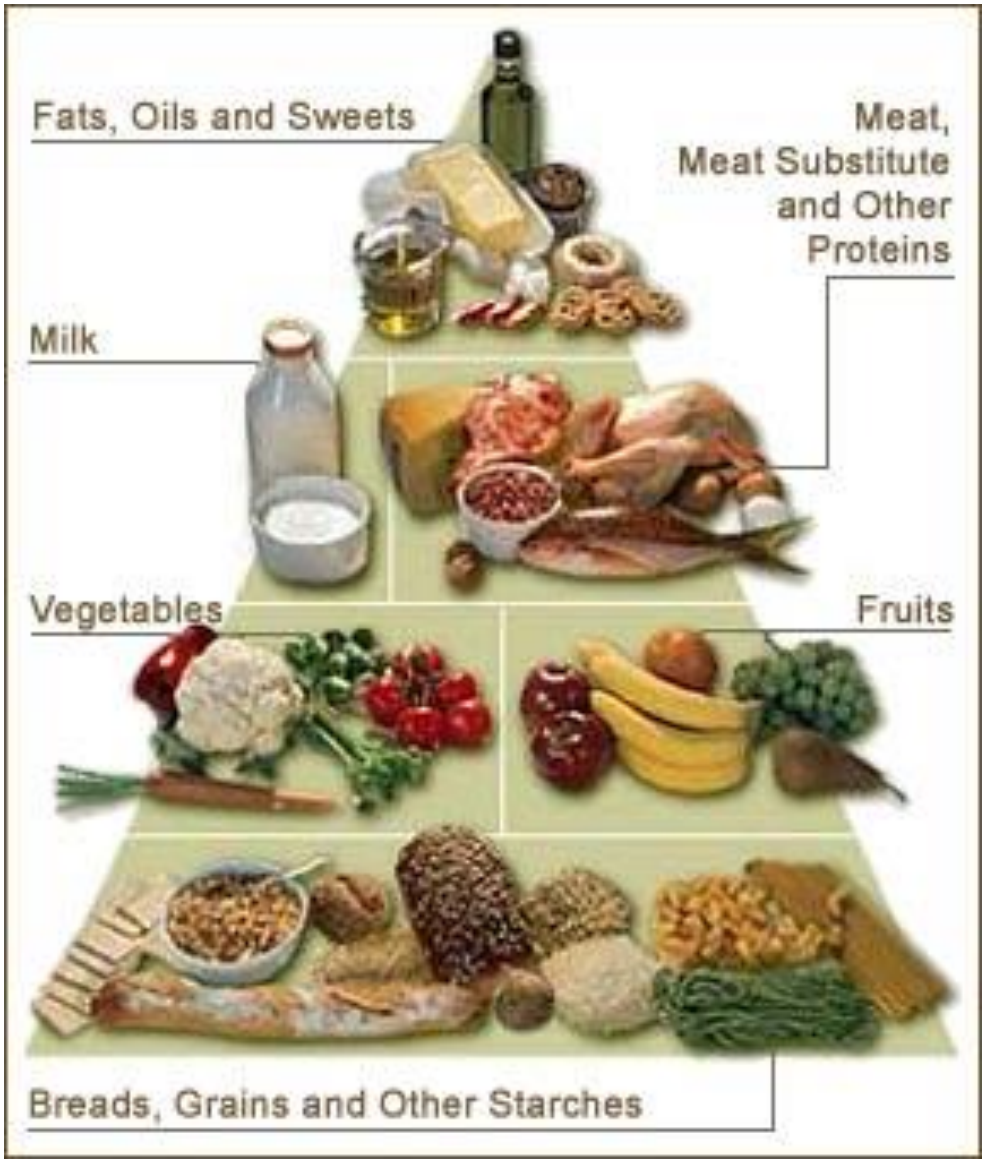


Figure 2: The Diabetes Food pyramid

Source: ADA (2009a)

In all, the carbohydrates with high glycaemic values are replaced with fibres and grains, more fruits, vegetables, proteins (with meat and meat substitutes and a little milk) and less saturated and trans fats and cholesterol intake (Fuller *et al.,* 2015; Liu *et al.,* 2015). Also, physical activity of 150 minutes per week with no more than 2 consecutive days without training is recommended (Hordern *et al.,* 2012).

2.7.2 Insulin Therapy

Insulin is an anabolic hormone that acts on various target tissues, including the liver, skeletal muscle, and fat tissue, regulating the blood glucose level (Bogan, 2012)**.** Exogenous insulin improves the body's ability to metabolize carbohydrate, store glucose in the liver as glycogen, and fat in the adipocytes (Lilley *et al*., 2014). While type 1 depends largely on insulin, type 2 is managed by the combination of insulin and oral agents (Garber *et al*., 2015).

Insulin initiates its action by binding to a glycoprotein receptor on the surface of the cell. This receptor consists of an X-subunit, which binds the hormone, and a J1-subunit, which is an insulin-stimulated, tyrosine-specific protein kinase. Activation of this kinase generates a signal that eventually results in insulin's action on glucose, lipid, and protein metabolism (Matveyenko *et al.,* 2012).

Insulin can be classified as rapid-acting, short acting, intermediate-acting or long-acting based on their pharmacokinetic profile (Matveyenko *et al.,* 2012)

2.7.3 Oral Antihyperglycemic Agents

2.7.3.1 Biguanides

Biguanides such as metformin reduce hepatic glucose production, decrease gastrointestinal glucose absorption, increase target cell insulin sensitivity and suppress hepatic glucagon signaling by decreasing production of cyclic adenosine monophosphate (AMP) (Lamanna *et al.,* 2011; Miller *et al.,* 2013).

Metformin is the first-line agent of use in type 2 diabetics where dietary modification cannot adequately control the glycaemic level. Its administration results in reduction of hepatic gluconeogenesis by inhibiting mitochondrial respiratory-chain complex 1 leading to activation of the AMP kinase–FOX03 pathway, reducing fatty acid oxidation and reactive oxygen species (Hou *et al.,* 2010). This improves the bioavailability of nitric oxide, endothelial function, and vascular blood flow (Triggle and Ding, 2010). Metformin is dominantly used in the management of type 2 diabetes due to its favourable and robust effects on cardiovascular risk (Anabtawi and Miles, 2016). Metformin side effects include nausea, vomiting, diarrhoea, reduction in circulating levels of vitamin B12, and megaloblastic anemia (Reinstatler *et al.*, 2012).

2.7.3.2 Insulin Secretagogue

Insulin secretagogues are oral antihyperglycemic agents that improves insulin secretion and sensitivity. There are two kinds of secretagogue drugs: sulfonylureas and non-sulfonylureas. Sulfonylurea drugs are those agents that stimulate pancreatic beta-cell insulin secretion, such as tolbutamide, chlorpropamide and carbutamide (Watson and Dokken, 2014) while non-sulfonylurea insulin secretagogues are newer agents that also stimulate insulin secretion but are more short-acting. Examples include glipizide, glibenclamide, gliclazide, glibornuride and glimepiride (Hellman, 1974; Hellman *et al.*, 1976; Papich, 2015).

Sulfonylurea was developed in 1966 in a cooperative study between Boehringer Mannheim (now part of Roche) and Hoechst (now part of Sanofi-Aventis) (Balsells *et al.*, 2015). It is used in the treatment of type 2 diabetes. As of 2003, in the United States, it was the most popular sulfonylurea (Riddle, 2003). These drugs stimulate insulin release through the closure of ATP-sensitive potassium channels on β-cells (Bryan *et al.,* 2005). While effective in controlling glucose levels, their use is associated with modest weight gain and risk of hypoglycemia. In addition, studies have demonstrated a secondary failure rate that

may exceed other drugs, ascribed to an exacerbation of islet dysfunction (Kahn, *et al.*, 2006). Shorter-acting secretagogues, the meglitinides (or glinides), stimulate insulin release through similar mechanisms but may be associated with less hypoglycemia and require more frequent dosing (Gerich, *et al.*, 2005).

2.7.3.3 Thiazolidinediones

Thiazolidinediones (TZDs) are peroxisome proliferator–activated receptor γ activators (Yki-Järvinen, 2004) that improve insulin sensitivity in skeletal muscle and reduce hepatic glucose production (Bailey and Turner, 1996; Lamanna *et al.*, 2011). They do not increase the risk of hypoglycemia and may be more durable in their effectiveness than sulfonylureas and metformin (Kahn, *et al.*, 2006). Pioglitazone, a member of thiazolidinediones, appeared to have a modest benefit on cardiovascular events in patients with overt macrovascular disease (Dormandy *et al.*, 2005). Recognized side effects of TZDs include weight gain, fluid retention leading to edema and/or heart failure in predisposed individuals, and increased risk of bone fractures (Dormandy *et al.*, 2005; Kahn, *et al.*, 2006).

2.7.3.4 α-glucosidase Inhibitors

α-glucosidase inhibitors (AGIs) such as acarbose and miglitol are oral antidiabetic agents that specifically inhibit α-glucosidases in the brush border of the small intestine (Ghosh and Collier, 2012). AGIs act by delaying the breakdown of complex starches to oligosaccharides. The delay in postprandial glucose peaks that results eventually leads to decreased post-load insulin levels. Known side effects of this class of drug include flatulence, diarrhoea and stomachache (Ghosh and Collier, 2012).

2.7.3.5 Glucagon-like Peptide-1 Agonists

Glucagon-like Peptide-1 (GLP-1) is an intestinally derived incretin hormone that potentiates the glucose metabolism-dependent secretion of insulin from the beta cells (Hölscher, 2010). Example of such drug is exenatide (Cacciatori *et al.*, 2017). It is secreted mainly by the intestine in a nutrient-dependent manner and stimulates glucose-induced insulin secretion, inhibits gastric emptying, food intake, and glucagon secretion (Lee and Jun, 2014). Although, GLP-1 enhances cAMP production when it binds to a specific GLP-1 receptor expressed in various tissues including pancreas, the exact pathway through which it exert its effects is not clear (Lee and Jun, 2014; Pyke *et al.*, 2014). Side effects of GLP-1 include nausea and vomiting (Deacon, 2011).

2.7.4 Lifestyle

Interventions on lifestyle are usually designed to impact an individual's physical activity levels and food intake as they are critical parts of diabetes management (Anderson *et al.*, 2003; Klein *et al.*, 2004). All patients should receive standardized general diabetes education (individual or group, preferably using an approved curriculum), with a specific focus on dietary interventions and moderate physical exercise including aerobic, resistance, and flexibility training (Hordern *et al.*, 2012).

Weight reduction, achieved through dietary means alone or with adjunctive medical or surgical intervention, improves glycemic control and other cardiovascular risk factors. Modest weight loss (5–10%) contributes meaningfully to achieving improved glucose control (Inzucchi *et al.*, 2015) while personalizing dietary advice (Bantle *et al.*, 2010).

2.7.5 Phytotherapy

Medicinal plants are moving from fringe to mainstream use with a greater number of people seeking remedies and health approaches free from side effects occasionally caused by

synthetic chemicals (Shivjeet *et al*., 2011). Some of these plants (Table 3) have been reported to have anti-diabetic activity (Afrisham *et al.,* 2015).

Extracts from these plants often improve the performance of pancreatic tissue, which is done primarily by increasing insulin secretion, reducing the intestinal absorption of glucose, increasing glucose absorption by muscle and fat cells, prevention of glucose absorption from the intestine and prevention of glucose production from hepatic cells (Hegazy *et al.,* 2013; Kooti *et al.,* 2016). They are the main source of organic compounds such as (polyphenols, tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids) which serve as a source for the discovery and development of new types of antidiabetic agents (Firdous, 2014). Many phytochemicals isolated from plant sources (Table 4) have shown antidiabetic activity with their mechanism of action elucidated and are now being used in managing diabetes in various parts of the worlds.

Many modern pharmaceuticals used in conventional medicine today also have natural plant origins. Among them is metformin derived from the flowering plant, *Galega officinalis* (Ikeda *et al.,* 2000). To this effect, a number of plants, herbal extracts, or phytochemicals broadly used as foods, dietary supplements and drugs (Santini and Novellino, 2014; Nasri *et al*., 2015) have been marketed for their health-promoting effects that has either been scientifically proven (as the case of *Galega officinalis*) or rely largely on the history and ethnopharmacological prospects (as the case of *Chrysophyllum albidum*) (Lapenna *et al*., 2015; Mocan *et al*., 2016).

Table 3: Plants scientifically validated for managing diabetes

Plant	Treatment	Effects	Reference
Punica granatum	Fruit/concentrated juice	Decreases TC, LDL, atherosclerotic index and no significant effect on TRIG and HDL	Esmaillzadeh *et al* (2004)
Portulaca oleraea	Seed/powder	Decreases FBG, TC, TRIG, LDL, total and direct bilirubin, BMI, Body weight, insulin, hepatic ALT, AST and GTT and increases HDL	El-Sayed (2011)
Vitis vinifera	Seed/ethanolic extract	FBG, SOD, GPx, MDA of diabetic treated the extract compared well with the placebo group	Pourghassem-Gargari *et al* (2011)
Vitis vinifera	Fruit/resveratrol	Decreases FBG, oxidative stress and increases insulin sensitivity index, platelet Akt phosphorylation, urinary ortho-tyrosine excretion but no effect on beta cell function and serum insulin level	Sharma *et al* (2008)
Coriandrum sativum L.	Fruit/powder	Decreases FBG, TC, TRIG,LDL, atherosclerotic index and increases cardioprotective index	Parsaeyan (2012)
Portulaca oleraea	Seed/powder	Increases glucagon-like peptide-I concentration but no effect on glucagon-like peptide-I receptor	Heidarzadeh *et al* (2013)
Vitis vinifera	Fruit/polyphenol extract	increases hepatic insulin sensitivity index, mitochondrial respiration and decreases glucose infusion rate, oxidative stress	Hokayem *et al* (2013)

Abbreviations: FBG, fasting blood glucose; TC, total cholesterol; LDL, low density lipoprotein; TRIG, triglyceride; HDL, high density lipoprotein; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transferase; SOD, superoxide dismutase; GPx, glutathione peroxidase; MDA, malondialdehyde; Akt, protein kinase B.

Table 4: Phytochemicals for the Management of Diabetes

Phytochemical	Source	Mechanism of Action	Reference
Marrubin	*Leonotis leonurus*	Increases the insulin level and glucose transporter-2 gene expressions in INS-1 cells	Mnonopi *et al* (2012)
Chalcomoracin, Moracin C, Moracin D and Moracin N	*Morus alba*	Inhibits α-glucosidase activity	Yang *et al* (2012)
1,2,3,4,6 Penta-O-galloyl-β-d-glucose	*Mangifera indica*	Inhibits 11-β-HSD-1 and ameliorates high fat diet-induced diabetes in C57BL/6 mice	Mohan *et al* (2013)
8-oxo-berberine	*Berberis brevissima*	Inhibits Protein Tyrosine Phosphatase 1B (PTP 1B)	Ali *et al* (2013)
Ginsenoside Re	*Panax ginseng*	Reduces insulin resistance through activation of PPAR-γ pathway and inhibition of TNF-α production	Gao *et al* (2013)
Procyanidins	*Theobroma cacao*	promote glucose uptake activity by inducing GLUT4 translocation through AMPK-dependent pathway in muscle	Yamashita *et al* (2012)

2.8 *Chrysophyllum albidum*

African star apple botanically called *Chrysophyllum albidum* (plate 1), belongs to the Sapotaceae family and grows up to 25 to 37 m high (Adebayo *et al.,* 2010). It is a tropical fruit tree that is widely distributed in the lowland rain forest zones of Africa (Madubuike and Ogbonnaya, 2003). It is called *agbalumo* (Yoruba), *udara* (Igbo), *agbaluba* (Hausa) and *eha* (Ebira) in Nigeria local languages (Orijajogun *et al.*, 2013). The tree produces a sweet round edible fruit that has a single hard pit and varies in colour from bright red or yellow to dark purplish black and has various ethno-medical uses (Amusa *et al*., 2003).

The plant parts are used locally for management of various disease conditions. The leaf is used as an emollient and for the treatment of stomachache and diarrhoea. The leaf and cotyledons from its seed are used as ointments in the treatment of vaginal and dermatological infections in Western Nigeria (Adewusi, 1997). The roots, barks and leaves of *C. albidum* are widely used as an application to sprains, bruises and wounds in southern Nigeria. The seeds and roots extracts are used to arrest bleeding from fresh wounds, and to inhibit microbial growth (Okoli and Okere, 2010) while the stem bark is used for the treatment of yellow fever and malaria (Florence and Adiaha, 2015).

Adebayo *et al* (2011) investigated the lipid peroxidation, antioxidant and free radical scavenging properties of aqueous, petroleum ether, ethanol, butanol and ethylacetate leaf extracts of *Chrysophyllum albidum* by employing the *in vitro* and *in vivo* experimental models and concluded that *Chrysophyllum albidum* could be employed as natural antioxidant boosters for the treatment of free radical implicated oxidative stress disorders. Imaga and Urua (2013) compared the aqueous and ethanolic extract of the fruit and concluded that the ethanolic extract of the fruit showed greater scavenging activity at all concentrations.

Leaf Fruit Stem bark

Plate 1: ***Chrysophyllum albidum***

Idowu *et al* (2006) isolated eleagnine, an alkaloid, from *C. albidum* seed cotyledon and reported that it has antioxidant activity. Olorunnisola, *et al* (2013) reported the hypoglycemic and hypolipidemic effect of ethanolic extract of *Chrysophyllum albidum* seed cotyledon in alloxan-induced diabetic rats while Onyeka *et al* (2012) reported the anti-hyperglycaemic, hepatoprotective and free radical scavenging activity of the ethanolic root bark extract of *Chrysophyllum albidum* in alloxan-induced diabetic rats. The plant has also been reported to have antimicrobial (Duleyemi and Lawal, 2009; Okoli and Okere, 2010; Ajetunmobi and Towolawi, 2014; MacDonald *et al.,* 2014) and antiplasmodial (Adewoye *et al.*,2010, 2013) activities. The antifatility activity of the ethanolic extract of the leaf and root stem bark has also been reported. While the leaf significantly increased the body and testis weight, motility, morphology and number of spermatozoa in caudal epididymis, superoxide dismutase activity, the root bark significantly decreased the parameters and the hormonal (follicle stimulating hormone, luteinizing hormone and testosterone) levels in both extracts significantly decreased (Onyeka *et al.,* 2012, 2013).

The various pharmacological activities shown by the plant are due to the presence of secondary metabolites that include alkaloids, tannin, saponin, phenol and flavonoid (Okoli and Okere, 2010). However, the acclaimed folkloric use of *C. albidum* stem bark in the management of diabetes is yet to be verified and hence the need for this study.

2.9 Induction of Experimental Diabetes

Experimental animal model of a disease does not only aid in understanding the pathophysiology of such disease, but also in the development of drugs for its management (Etuk, 2010). Several models of inducing animals into diabetes have been established and include chemical, surgical and genetic manipulations (Srinivasan and Ramarao, 2007). One of the chemical methods of inducing experimental diabetes mellitus is the use of Alloxan.

Alloxan (Figure 3) (2,4,5,6-tetraoxypyrimidine;2,4,5,6-pyrimidinetetrone), an oxygenated pyrimidine derivative, is a glucose analogue that fix and accumulate to the pancreatic beta cells via the GLUT2 glucose transporter and selectively impedes glucose-induced insulin secretion through specific inhibition of glucokinase which causes a state of insulin-dependent diabetes due its ability to induce reactive oxygen species development, resulting in the discerning necrosis of the pancreatic beta cells (Lenzen, 2008).

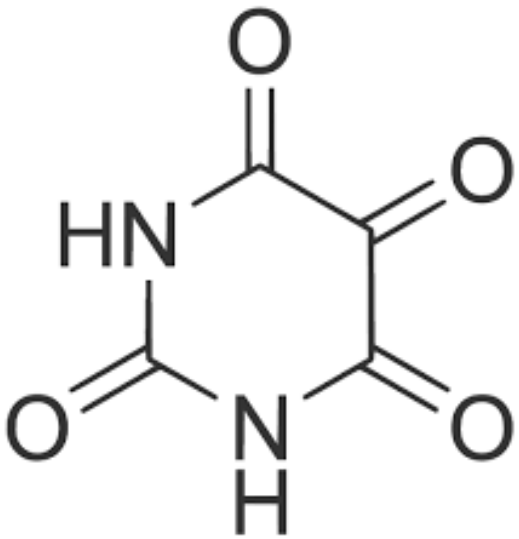


Figure 3: Structure of Alloxan

Source: Gorus *et al* (1982)

It exercises its diabetogenic action when administered intravenously, intraperitoneally or subcutaneously. Furthermore, the dose of alloxan required for inducing diabetes depends on the animal species, route of administration and nutritional status (Federiuk *et al*., 2004). Moreover, alloxan has been established to be non-toxic to the human beta-cells, even in very high amounts, the reason of which may be ascribed to the differing glucose uptake mechanisms in humans and rodents (Eizirik *et al*., 1994; Tyrberg *et al*., 2001).

Among several researchers that have used alloxan-induced diabetic animals as a model for studing diabetes include Kazemi *et al* (2010), Viswanathaswamy *et al* (2011), Rohilla and Shahjad (2012), Jarald *et al.* (2013), Obeten *et al.* (2014), Yakubu and Ogunro (2014), El-Desouki *et al* (2015), Amraie *et al* (2015), Farsani *et al* (2016), Cheekati *et al* (2017) and Ibrahim *et al* (2018).

2.10 Phytochemicals

2.10.1 Procyanidins

Procyanidins (Figure 4) are a subclass of flavonoids and are composed of the flavan-3-ol monomers that naturally occur in plants. They are known to exhibit many physiological activities including antioxidant, cardioprotective, anti-inflammatory, anti-hyperglycaemic and enzyme inhibitory effects (Hooper *et al.,* 2008; Yamashita *et al.,* 2012; Martin *et al.,* 2013). Various studies have shown that health benefits of procyanidins are related to their structures (Bryan *et al.,* 2002; Chen *et al.,* 2012; Dorenkott *et al.,* 2014), and the structural diversity is due to the type of interflavanoid linkage, the kind and number of flavan-3-ol units. Most oligomeric proanthocyanidins (OPCs) and polymeric proanthocyanidins (PPCs) are linked through C4 → C8 and/or C4 → C6 interflavan bonds as (+)-catechin and/or (−)-epicatechin as basic unit (Lia *et al.,* 2016). Procyanidins (Figure 4) are found in various plant-derived foods, such as apple, pear, berries, wine, cacao, and nuts (Sun and Spranger, 2005; Pérez-Jiménez *et al.,* 2010; Andre *et al.,* 2012). The presence of dimeric pro-cyanidins B1 to B7,

trimeric procyanidin C1, tetrameric procyanidin(cinnamtannin A2) and pentameric procyanidin (cinnamtannin A3) in cocoa products has been reported (Kothe *et al.,* 2013; Esatbeyoglu *et al.,* 2015). The antidiabetic mechanisms of procyanidins depends on the degree of polymerization as low-degree polymerization of procyanidins promote glucose uptake in the muscle by inducing GLUT4 translocation through the AMPK-dependent pathway, while high- degree polymerization of procyanidins inhibit α-glucosidase and thus delay glucose absorption in small intestine (Yamashita *et al.,* 2012).

2.10.2 Stigmasterol

Stigmasterol (Figure 5) is a member of the phytosterol family. It is present in plant oil and plant-based foods, such as soybean, corn, peanut, and sunflower oils (Li *et al.,* 2015). It is a bioactive compound present in plants that has a structure similar to cholesterol. It has no influence on total cholesterol and low density lipoprotein but decreases the atherogenic index (Abumweis *et al.,* 2008). It has a variety of pharmacological properties, including anti-osteoarthritic, anti-mutagenic, anti-inflammatory activity, anti-diabetic and anti-tumor properties (Gabay *et al.,* 2010; Kim *et.al.,* 2014; Zielińska and Matkowski, 2014; Casal *et al.,* 2016; Ramu *et al.,* 2016; Wang *et al.,* 2017). Stigmasterol isolated from banana peel exhibited a potential anti-diabetic effect in alloxan-induced diabetic rats (Ramu *et al.,* 2016). It has also been found to effectively ameliorate the hyperglycemia and hyperlipidemia in KK-Ay mice (Wang *et al.,* 2017). The possible antidiabetic mechanism of stigmasterol has been linked to its enhancement of GLUT4 expression and translocation *in vitro* and *in vivo* (Wang *et al.,* 2017).

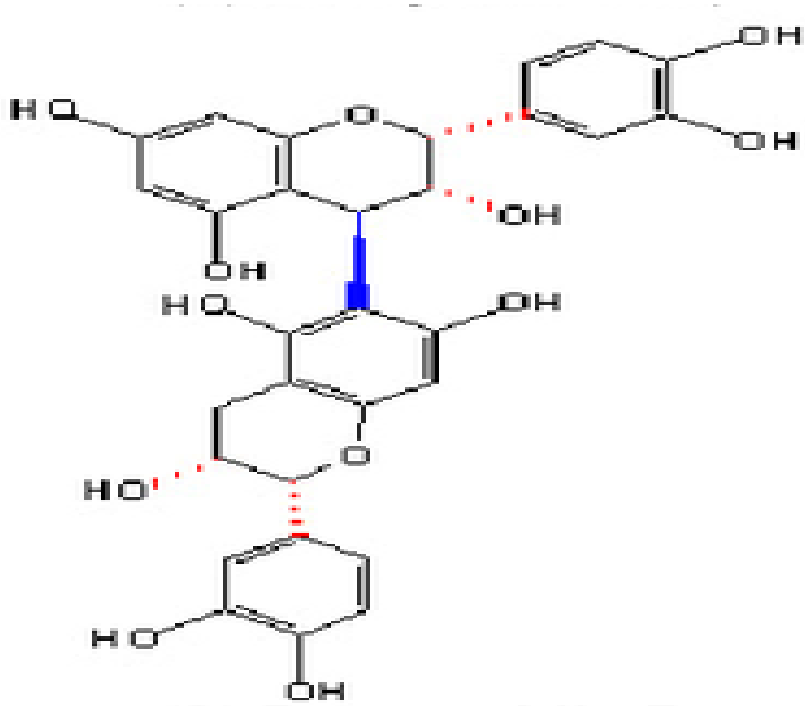


Figure 4: Structure of Procyanidin

Source: He *et al* (2008)

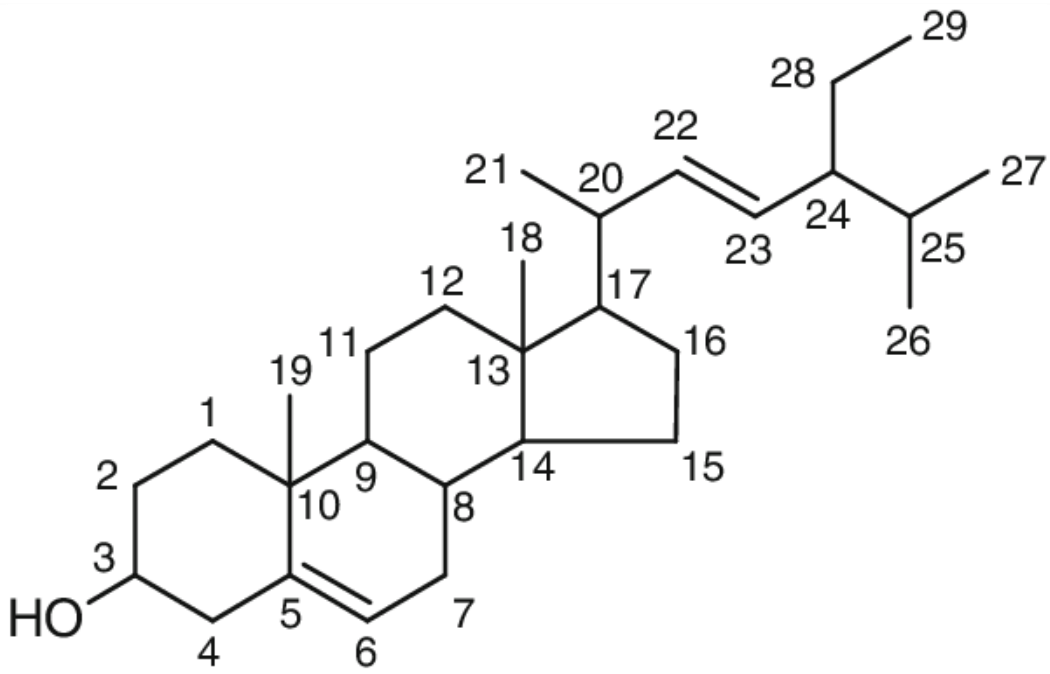


Figure 5: Chemical structure of stigmasterol (stigmasta-5,22-diene-3-ol)

Source: Struijs *et al* (2010)

2.10.3 Catechins

Catechins (or flavan-3-ols) composed of two aromatic rings linked to a dihydropyran heterocyclic ring and are characterized by the presence of several hydroxyl groups (Figure 6) (Dias *et al.,* 2014). They constitute the most abundant class of phenolic compounds found in unfermented teas. The major catechins found in green tea are (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-)-epigallocatechin 3-gallate (EGCG) (Moderno *et al.,* 2009; Dias *et al.,* 2014; Nunes *et al.,* 2014). These compounds have antioxidant power (Aboul-Enein *et al.,* 2013; Costa *et al.,* 2009), with epicatechin having not only a direct antioxidant effect but also able to conserve other antioxidants such as vitamins C and E (Auger *et al.,* 2004). Epigallocatechin is insulinomimetic, decreasing not only the production of glucose but also the expression of genes that control gluconeogenesis (Waltner-Law *et al.,* 2002). It also reduces expression of genes involved in fatty acids synthesis, downregulated genes involved in gluconeogenesis, and increased genes involved in glycolysis and glucose transporter 1 (GLUT 1) (Wolfram *et al.,* 2006). Catechins, mainly EGCG, and the aflavins help to prevent hyperglycaemia by enhancing insulin activity and possibly by preventing damage in pancreatic beta-cells (Anderson *et al.,* 2003).

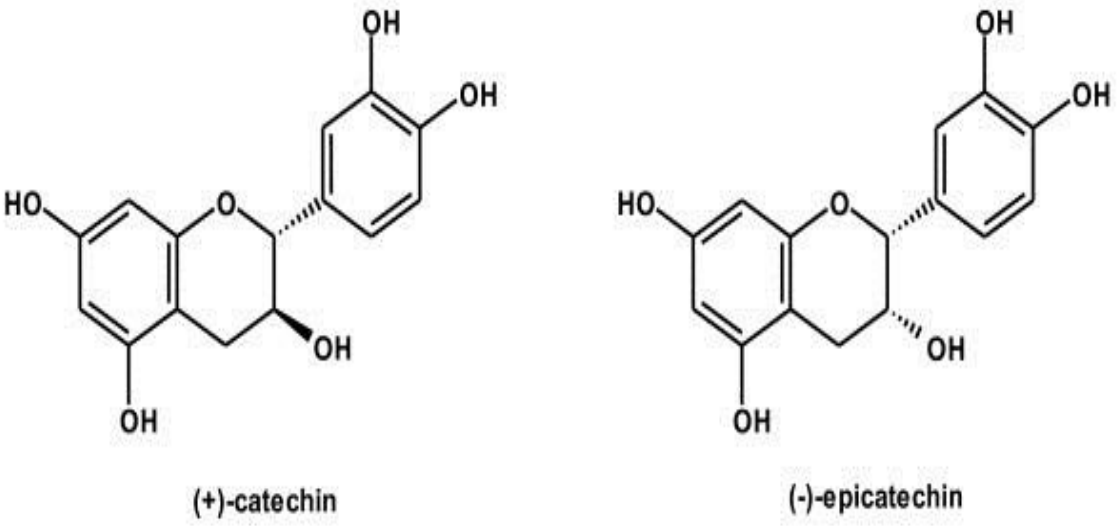


Figure 6: Structure of Catechin and Epicatechin
Source: Hurst *et al* (2011)

2.11 Organs Studied

2.11.1 The Liver

The liver is the second largest organ in the body (Vitt and Caldwell 2009). It is located in the upper right corner of the abdomen. The organ is closely associated with the small intestine, processing the nutrient-enriched venous blood that leaves the digestive tract (Plaats, 2005).

The rat liver consists of 4 distinct lobes (Figure 7); the left lateral lobe (which represents about 30% of the total liver weight), the right liver lobe (which represents about 19% of the total liver weight), the median lobe (which represents about 40% of the total liver weight) and the caudate lobes (which represents 7% of the total liver mass) (Madrahimov *et al.*, 2006).

The liver plays a major role in the regulation of functions necessary for the maintenance of systemic metabolic homeostasis and as such keep the serum glucose concentrations within physiological limits. Glycogenolysis and gluconeogenesis increase plasma glucose, fatty acid oxidation, while lipogenesis provides free fatty acids. Insulin controls serum glucose concentrations and induces the transcription of most of the metabolic enzymes involved in hepatic glucose production and in glycogen synthesis (Foufelle and Ferre 2002). Glycogenolysis is activated to produce glucose during short period of fast, whereas is suppressed by insulin after food intake (Gastaldelli *et al.*, 2001).

2.11.2 Liver and Diabetes mellitus

During longer periods of fasting (12-18 hours), glycogen stores are depleted in the liver and, thus, gluconeogenesis is stimulated to maintain normal serum glucose concentration (Boden and Shulman, 2002). Insulin is the primary hormone promoting the storage of energy after carbohydrate meal and is secreted in response to nutrients into the portal circulation. High levels of insulin promote the fast-fed shift in hepatic carbohydrate metabolism, stimulating glycogen synthesis and suppressing both gluconeogenesis and glycogenolysis

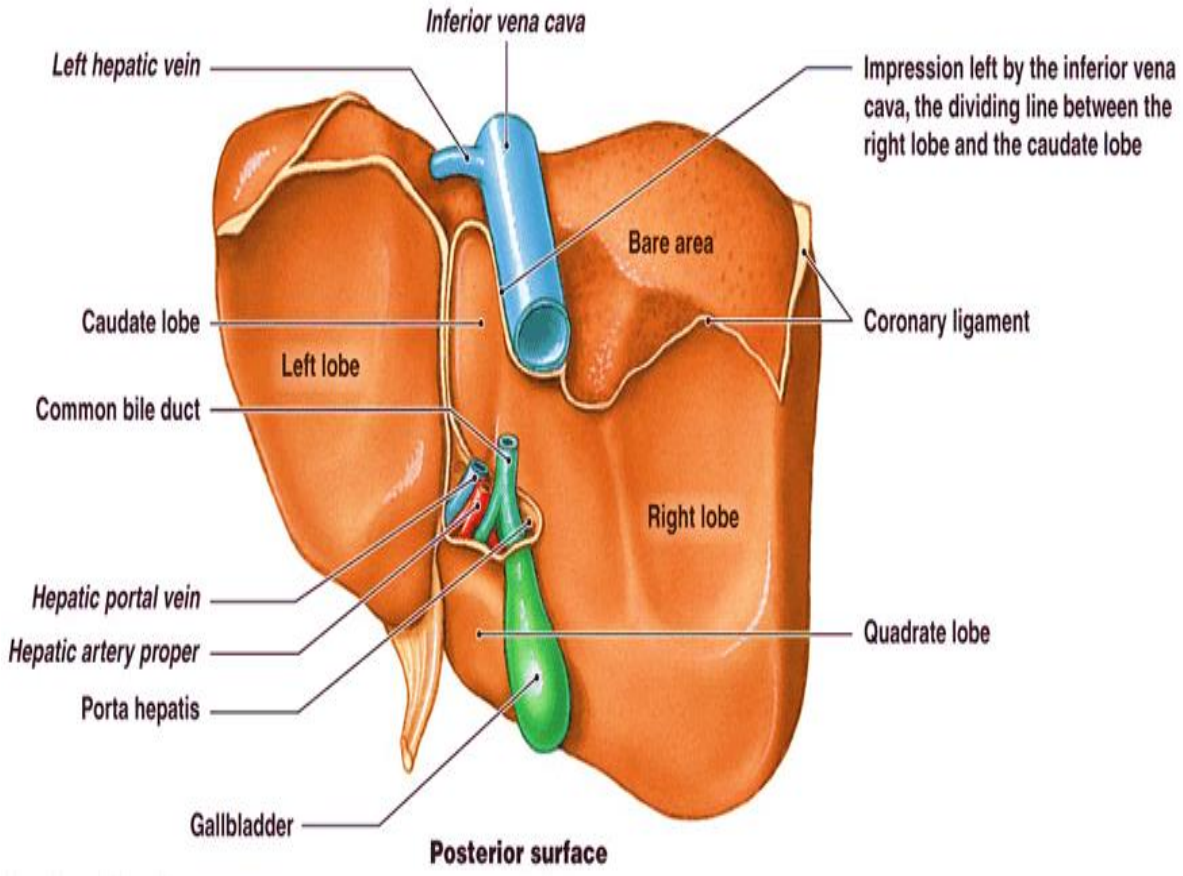


Figure 7: Anatomy of the Liver

Source: Kruepunga *et al* (2018)

In pathological states of absolute insulin deficiency, such as type-1-diabetes, or of relative insulin deficiency, such as obesity and type-2-diabetes, gluconeogenesis results, activated via an impaired inhibition of its key enzymes, phosphoenolpyruvate carboxykinase (PEPCK), responsible for the conversation of oxaloacetic acid to phosphoenolpyruvate, and glucose-6-phosphatase (G-6-Pase), responsible for dephosphorylation of glucose 6-phosphate to free glucose (Bischof *et al.,* 2002; Roden and Bernroider 2003). However, when the liver becomes fatty, insulin also fails to inhibit gluconeogenesis (Bugianesi *et al.*, 2005), causing an increase of serum glucose and, then, of hyperinsulinemia. Therefore, in a state of insulin resistance, the major alterations in glucose metabolism are represented by decreased ability of insulin to stimulate glucose consumption and to suppress hepatic glucose production (Figure 8) (D'Amore *et al.*, 2014).

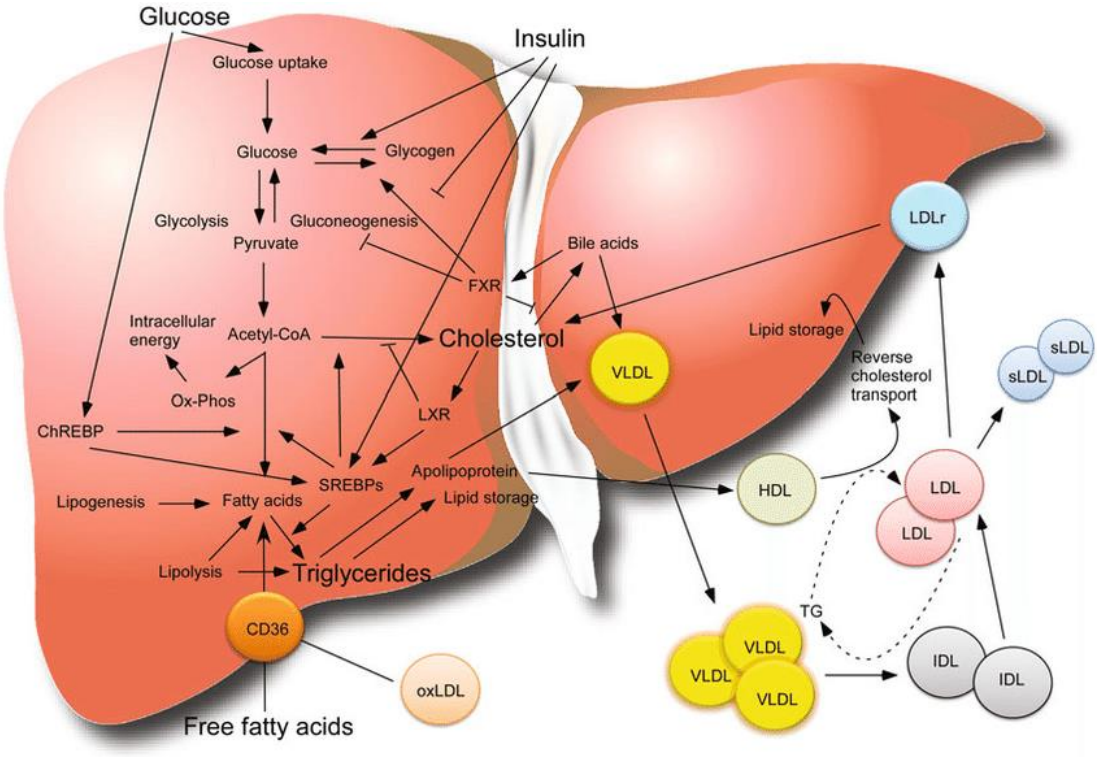


Figure 8: Role of the liver in the control of glucose and lipid metabolism

Source: D'Amore *et al.* (2014)

CD36 cluster differentiation protein-36, ChREBP carbohydrate response element- binding protein, FXR farsenoid X receptor, HDL high-density lipoprotein, IDL intermediate-density lipoprotein, LDL low-density lipoprotein, LDLr low- density lipoprotein receptor, LXR liver X receptor, OxLDL oxidized low-density lipoprotein, Ox-Phos oxidative phosphorylation, sLDL Small low-density lipoprotein, SREBPs sterol regulatory element binding proteins, TG triglyceride, VLDL very low-density lipoprotein

2.11.3 The Kidney

The kidneys are paired bean shaped organ, on each side of the spine with several functions. Lying at either side of the spine just below the diaphragm with adrenal gland resting on top of each kidney, the right kidney sits posterior to the liver while the left kidney sits posterior to the spleen (Walter, 2004; Carretero *et al,* 2017).

The left kidney is typically slightly larger than the right (Glodny *et al.,* 2009). The parenchyma of the kidney is divided into two major structures: superficial is the renal cortex and deep is the renal medulla. Grossly, these structures take the shape of 8 to 18 cone-shaped renal lobes, each containing renal cortex surrounding a portion of medulla called a renal pyramid (Walter, 2004). Between the renal pyramids are projections of cortex called renal columns. Nephrons, the urine-producing functional structures of the kidney, span the cortex and medulla. The initial filtering portion of a nephron is the corpuscle, located in the cortex, which is followed by a renal tubule that passes from the cortex deep into the medullary pyramids. Part of the renal cortex, a medullary ray is a collection of renal tubules that drain into a single collecting duct. The kidneys receive blood from the renal arteries, which branch directly from the abdominal aorta. Despite their relatively small size, the kidneys receive approximately 20% of the cardiac output (Walter, 2004).

The kidney (Figure 8) performs many sophisticated physiological processes and is largely responsible for maintaining the normal physiological balance of an individual (Sands and Verlander, 2005). Many of these functions are accomplished by relatively simple mechanisms of filtration, reabsorption, and secretion, which take place in the nephron. Filtration, which takes place at the renal corpuscle, is the process by which cells and large proteins are filtered from the blood to make an ultrafiltrate that will eventually become urine.

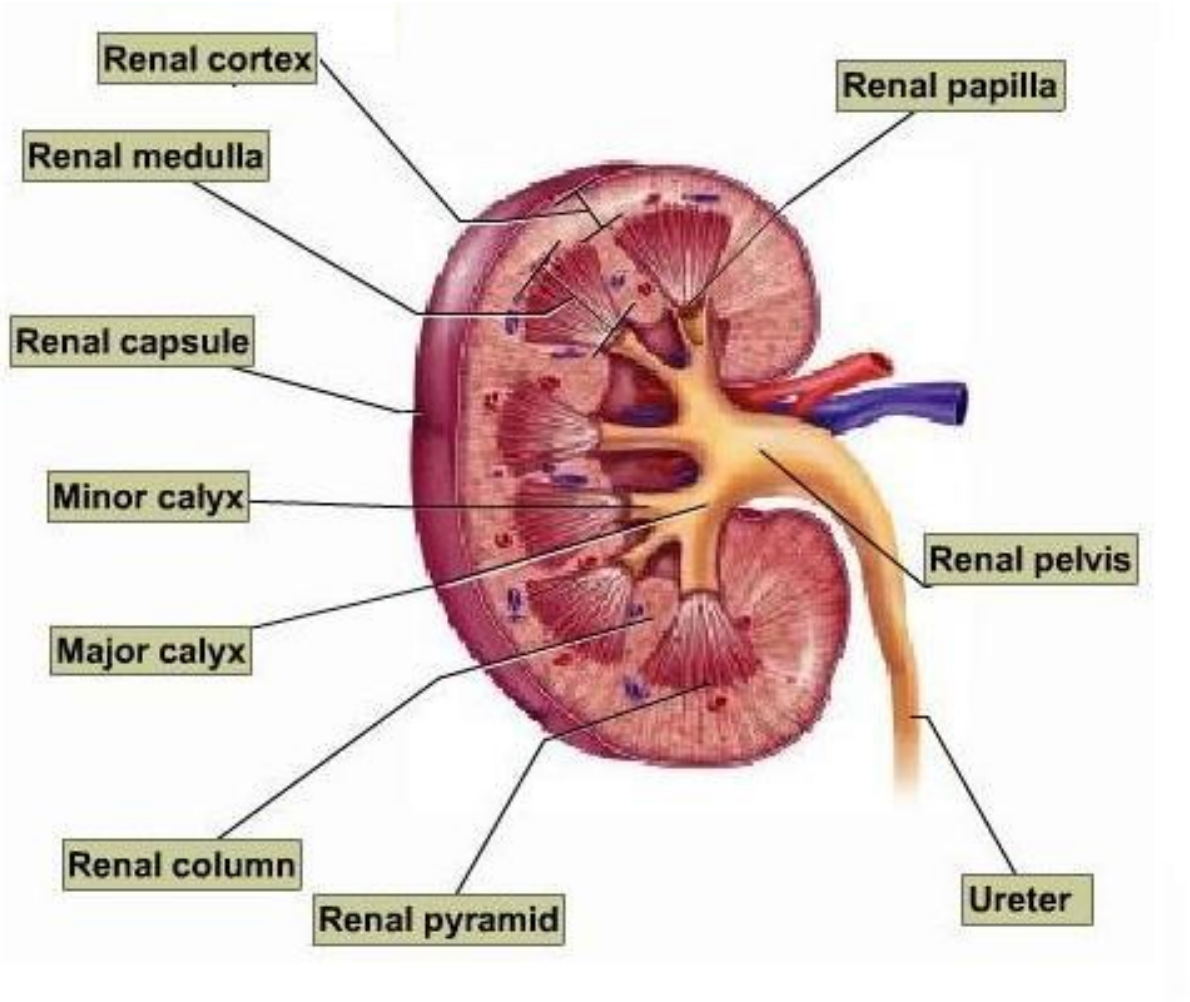


Figure 9: Anatomy of the kidney

Source: Patel (2009)

2.11.4 Kidney and Diabetes

Diabetes is the leading cause of death emanating from chronic kidney disease and distresses about 50% of diabetes population (NKF, 2012). Although efforts have been made to curb the menace, people with diabetes are still three times at risk of renal death than those without diabetes (Thomas *et al.,* 2016). As almost 25% of glucose from gluconeogenesis comes from the kidney, glucose reabsorption also increases due to persistent hyperglycaemia (DeFronzo *et al.,* 2012). This leads to mesangial extension, coagulating of the glomerular crypt membrane, and glomerular sclerosis. Thickening of the glomerular basement membrane may hamper filtration, and glomerular sclerosis that is branded by extracellular deposits within the glomerulus which reduces the surface area available for filtration. This aberration may cause cellular damage and eventually death (Soldatos and Cooper, 2008).

2.11.5 The Pancreas

The pancreas is a dual-function gland, having features of both endocrine and exocrine glands (Pandol, 2011). It is located behind the stomach within the left upper abdominal cavity and is partitioned into head, body and tail (Figure 9). It plays key roles in the regulation of macronutrient digestion and hence metabolism/energy homeostasis by releasing various digestive enzymes and pancreatic hormones. The majority of this secretory organ consists of acinar/exocrine -cells that secrete the pancreatic juice containing digestive enzymes, such as amylase, pancreatic lipase and trypsinogen, into the pancreatic duct. In contrast, pancreatic hormones are released in an endocrine manner, that is, direct secretion into the blood stream. The endocrine cells are clustered together, thereby forming the so-called islets of Langerhans, which are small, island-like structures within the exocrine pancreatic tissue that account for only 1–2% of the entire organ (Chandra and Liddle, 2009).

There are five different cell types releasing various hormones from the endocrine system: glucagon-producing α-cells (Brissova *et al.,* 2005), which represent 15–20% of the

total islet cells; amylin-, C-peptide- and insulin-producing β-cells (Brissova *et al.*, 2005), which account for 65–80% of the total cells; pancreatic polypeptide (PP)-producing γ-cells (Katsuura *et al.*, 2002), which comprise 3–5% of the total islet cells; somatostatin-producing δ-cells (Brissova *et al.*, 2005), which constitute 3–10% of the total cells; and ghrelin-producing ε-cells (Wierup *et al.*, 2002), which comprise <1% of the total islet cells.

Each of the hormones has distinct functions. Glucagon increases blood glucose levels, whereas insulin decreases them (Goke, 2008). Somatostatin inhibits both, glucagon and insulin release (Hauge-Evans *et al.*, 2009), whereas polypeptide regulates the exocrine and endocrine secretion activity of the pancreas (Batterham *et al.*, 2003; Katsuura *et al.*, 2002). Altogether, these hormones regulate glucose homeostasis in vertebrates. (Cabrera *et al.*, 2006; Brissova *et al.*, 2005).

2.11.6 Pancreas and Diabetes

The pancreas produces insulin to regulate glucose that might cause serious derangement in the body functions if left uncontrolled (Nalini *et al.*, 2018). It is released after meal to promote glucose utilization particularly for anabolic processes, and to discourage catabolism. Alteration in the production, availability or sensitivity of the hormone results in the alteration of energy homeostasis of the body. This leads to destruction of vital organs and may amount to complications and eventual death of the diabetic subjects (Reid *et al.*, 2012).

2.12 Enzymes Studied

2.12.1 Glucokinase (EC 2.7.1.2)

Glucokinase is a 50 KDa enzyme primarily responsible for the phosphorylation of glucose in the liver of mammals and other vertebrates. (Magnuson and Matschinsky, 2004).

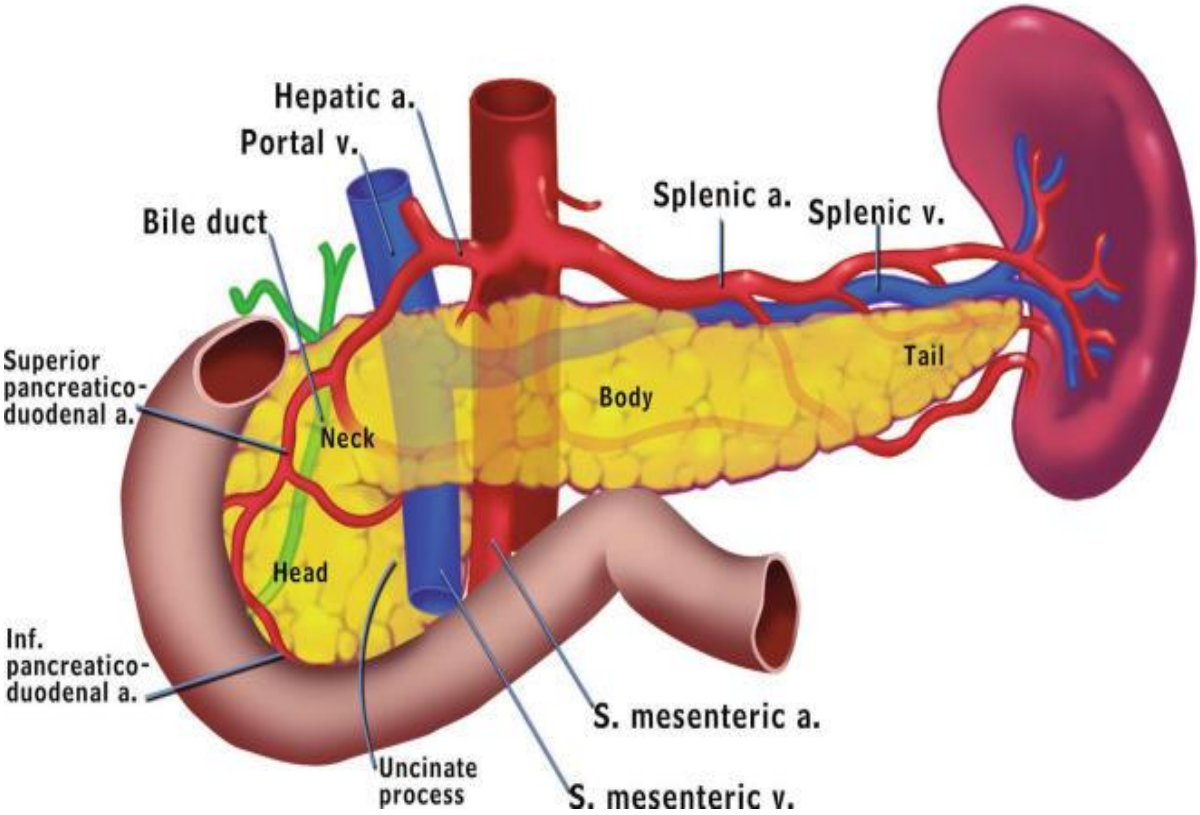


Figure 10: Anatomy of the pancreas

Source: Pandol (2011)

Distribution

Glucokinase is predominant in the hepatocytes of most but not all vertebrate but can also be found in pancreas, brain and the entero-endocrine K and L cells (Magnuson and Matschinsky, 2004)

Isoenzymes

Glucokinase is also known as hexokinase IV. The isoforms of this enzyme are hexokinases I, II and III (Kawai *et al.*, 2005).

Activators and Inhibitors

Glucokinase is activated by glucose, fructose-1-phosphate, insulin and glucokinase activator drugs while being inhibited by glucagon, fructose-6-phosphate and the liver-specific glucokinase regulatory protein (Zelent *et al.*, 2011).

Clinical Applications

Glucokinase functions as a glucose sensor in pancreatic β-cells and as a mediator of hepatic glucose disposal. These properties accord well with the presumed function of glucose phosphorylation in the liver as a means of controlling the blood glucose concentration as diabetes mellitus appears to be related to a decrease in glucokinase activity in the liver (Polonsky and Burant, 2016).

2.12.2 Glucose-6- Phosphatase (EC 3.1.3.9)

Glucose-6- phosphatase (G6Pase) complex catalyzes the terminal step of gluconeogenesis by dephosphorylating the glucose-6- phosphate to glucose (Nordlie *et al.*, 1999). G6Pase also dephosphorylates the glycogenolysis-derived glucose-6- phosphate to glucose and released into blood circulation in the same manner as that is produced by glucogenesis (Hers, 1976).

Distribution

Glucose-6- phosphatase is found in the liver, pancreas, intestine, muscle and in low concentration in the kidney (van Schaftingen and Gerini, 2002).

Activators and Inhibitors

Glucose-6- phosphatase is activated by glucagon and cyclic adenosine monophosphate and inhibited by calcium and insulin (Li and van de Werve, 2000).

Clinical Applications

Glucose-6-phosphatase is commonly used for diagnosing Von Gierke disease and glycogen storage disease type 1 that is characterized by accumulation of glycogen and fat resulting in hepatomegaly and renomegaly (Froissart *et al.,* 2011).

2.12.3 Fructose 1, 6-bisphosphatase (EC 3.1.3.11)

Fructose-1,6-bisphosphatase is one of the key enzymes of gluconeogenesis where it catalyses the conversion of fructose-1,6-bisphosphate to fructose-6-phosphate. Increase activity of this enzyme has been found to increase glucose production during diabetes mellitus (Pari and Chandramohan, 2017).

Distribution

Fructose-1,6-bisphosphatase is found in the liver, kidney and skeletal muscle. The liver form plays a critical role in maintaining blood glucose levels both in liver and the kidney where it has been the predominant source of de novo synthesized glucose (gluconeogenesis) while in kidney, a relatively minor source of de novo synthesized glucose except during periods of extreme fasting and/or in the diabetic state (Gerich *et al.,* 2005).

Izoenzymes

Two isoforms of Fructose-1,6-bisphosphatase has been reported in mammals namely, liver fructose-1,6-bisphosphatase (FBPI), which is expressed primarily in liver and kidney,

and the muscle Fructose-1,6-bisphosphatase (FBP2), which is found exclusively in muscle tissue (Nishimasu *et al.,* 2004).

Activators and Inhibitors

Fructose-1,6-bisphosphatase is activated by adenosine triphosphate and Fructose-1,6-bisphosphate in the presence of divalent cations such as magnesium, manganese or zinc and inhibited by cyclic adenosine monophosphate and Fructose-2,6-bisphosphate (Chou *et al.*, 2009).

Clinical Applications

Fructose-1,6-bisphosphatase has found application in the management of diabetes as alteration of its activity can have a large effect on gluconeogenesis while reducing the risk of hypoglycemia and other potential side effects from altering other enzymes in gluconeogenesis (Dang *et al.,* 2012).

2.12.4 Glucose 6-phosphate Dehydrogenase (EC 1.1.1.49)

Glucose 6-phosphate dehydrogenase is a ubiquitous enzyme, which is critical in the redox metabolism of all aerobic cells (Sodiende, 1992). It is the first and rate-limiting enzyme of the pentose phosphate pathway, which results in the production of ribose-5-phosphate and NADPH (Xu *et al.,* 2005).

Distribution

Glucose 6-phosphate dehydrogenase is found in many tissues but its concentration is very high in liver and mammary gland (Schnarrenberger *et al.,* 1973).

Izoenzymes

Two major izoenzymes of glucose 6-phosphate dehydrogenase known are G6PDH I and G6PDH II (Hong and Copeland, 1991).

Activators and inhibitors

The enzyme is activated by $NADP^+$ glucose-6-phosphate in presence of Mg^{2+} and inhibited by NADPH and ATP (Schnarrenberger *et al.*, 1973).

Clinical Applications

Glucose-6-phosphate dehydrogenase assays has found application in monitoring oxidative stress implicated diseases and in diagnosing drug induced hemolytic anaemia often called "primaquine sensitivity" (Buritis and Ashwood, 1999).

2.12.5 Alkaline Phosphatase (E.C. 3.1.3.1.)

Alkaline phosphatase is a substrate non-specific phosphomonoestrase which exist in all living forms from bacteria to human and displays optimum activity between pH 9 and 10 (Ozer *et al.*, 2008). ALP acts on a large variety of natural and synthetic substrates (Guyton and Hall). Commonly use substrates for determining its activity are 3-glycerophosphate, phenyl phosphate and paranitrophenyl phosphate (Mazzei *et al.*, 2004). It is activated by some divalent ionsincluding Mg^{2+}, Mn^{2+} and Co^{2+} and inhibited by Zn, Be, Cu, Hg ions, sulphide, cyanide, adrenaline, oxalate, borate, adrenochrome and oxidizing agents (Millan, 2006). The exact metabolic function of alkaline phosphate is not known but it is probably important in calcification of bone (Phillip *et al.*, 1994).

Distribution

Alkaline phosphatase is present in most tissue but particularly of high concentration in the bone osteoblasts, hepatobiliary tract, intestinal wall, heart, renal tubules and placenta (Kaplan, 1972).

Isoenzymes

Studies have identified different forms of alkaline phosphatase in animal models and are different in their amino acid constituents. It can be found in the liver, kidney, mammary gland, intestine among other tissues (Dzoyem *et al.*, 2014).

Activators and Inhibitors

Alkaline phosphatase is activated by some divalent ions such as Mg^{2+}, Mn^{2+} and Co^{2+} (Millan, 2006). Optimal activity being obtained with Mg^{2+} at about 1.0mol/L. However, glycine and alanine at low concentrations increase serum ALP activity. It is inhibited by Zn, Be, Cu, Hg ions, sulphide, cyanide, adrenaline, oxalate, borate, adrenochrome and oxidizing agents (Millan, 2006).

Clinical Applications

Alkaline phosphatase is a marker enzyme for endoplasmic reticulum (Wright and Plummer, 1974) and is frequently used to assess the integrity of the plasma membrane (Akanji *et al*., 1993). ALP is released into the blood in various disorders, including obstructive jaundice and some bone diseases. Measurement of it level in serum forms a common clinical test. Serum alkaline phosphatase measurement is a useful clinical test in diagnosing hepatobiliary (Singh *et al.,* 2011) and bone cholestasis (Ramaiah, 2007).

2.12.6 Alanine Aminotransferase (E.C. 2.6.1.2)

Alanine aminotransferase (ALT) generally referred to as Glutamate pyruvate transaminase has its systematic name as L-alanine-2-oxoglutarate aminotransferase and catalyzes the transamination reaction between L-alanine and α-ketoglutarate to form pyruvate and oxaloacetate (Dzoyem *et al.*, 2014).

Distribution

ALT is less abundant than AST in human tissues (Wilkinson, 1970), it is native to the cytosol, though small amount have been found in the mitochondria of liver cells and also in the heart muscles (Yang *et al.,* 2004).

Isoenzymes

The isoenzymes of ALT show variation in their rate of reaction with substrates and denaturation by heat. The cytoplasmic and mitochondrial fraction of the ALT shows the same

pH sensitivities and immunological properties, though they can be distinguished by their differences in electrophoretic mobilities (Reitman and Frenkel, 1957). The activity of alanine aminotransferase is stimulated by a high concentration of aldehydes, oxoacids and ketones (Schmidt *et al*., 1967) and inhibited by the accumulation of one of the reaction products, pyruvate (Reitman and Frenkel, 1957). The activities of ALT are measured as one of the marker traceable to changes in the pathological condition of the liver. The level of serum ALT can be used as a differential diagnosis of liver and heart diseases (Reitman and Frenkel, 1957).

Activators and Inhibitors

The activity of alanine aminotransferase is stimulated by a high concentration of aldehydes, oxoacids and ketones (Schmidt *et al*., 1967). It is inhibited by the presence of accumulated pyruvate which is one of the reaction products (Reitman and Frenkel, 1957).

Clinical Applications

Increase in serum ALT is considered more sensitive indicator of hepatitis and liver cell damage than AST, as the former is found in high concentrations in the liver than in the heart muscles (Reitman and Frenkel, 1957). Schmdt *et al* (1967) applied ALT to the diagnosis of liver diseases, high levels of ALT are observed in patients with acute hepatitis and acute cirrhosis, while it might not be detected in sera of healthy subjects. The AST/ALT ratio of serum sample is useful in differential diagnosis of jaundice (Reitman and Frenkel, 1957).

2.12.7 Aspartate Aminotransferase (E.C. 2.6.1.1)

Aspartate aminotransferase (AST) generally referred to as Glutamate oxaloacetate transaminase (George *et al*., 1976) and has its systemic name as L-aspartate-2-oxoglutarate aminotransferase. It catalyzes the transamination reaction between L-aspartate and α-ketoglutarate to form glutamate and oxaloacetate at optimum pH of 7.4 with the liver, heart, skeletal, muscle and kidney being the richest sources (Wilkinson, 1970).

Distribution

AST is widely distributed in animal tissues with a relatively high concentration in the heart muscle (Bhargava and Screenivasan, 1965). The liver also contain considerable amount of this enzyme (Wilkinson, 1970). It is also found in the kidney, pancreas, lung and spleen also contain considerable quantities of the enzyme. It is found both in the cytosol and in the mitochondria of the cells (Boyd, 1961).

Isoenzymes

There are two isozymes of AST, isozymes I and II, cationic and anionic respectively. They differ in their electrophoretic mobilities, pH variation, immunochemical properties and degree of substrate affinities. They both originate from the mitochondria (Boyd, 1961). Isozyme I (cationic isozymes) has a much greater affinity for aspartate than isozyme II (anionic isozymes) (Boyd, 1961).

Activators and Inhibitors

The activities of AST are inhibited by its product, oxaloacetate. Therefore, it is an allosteric enzyme. The activator of AST is pyridoxal phosphate which act as a cofactor in the presence of Mg^{2+} (Nelson and Cox, 2000).

Clinical Applications

It is used in the diagnosis of liver diseases caused by drug toxicity or by infection (Nelson and Cox, 2005). High level of mitochondrial AST has been observed in the sera of patients having acute and chronic cirrhosis (Schmidt *et al.*, 1967).

2.13 Liver and Kidney Function Indices

There is no single test for measuring liver and kidney function, because all the functions of the liver are not equally or simultaneously affected in hepatobilliary or renal disorder. Consequently, a battery of tests assessing a variety of functions has to be carefully chosen. The concentration of proteins, bilirubin and albumin in the serum can be used to

ascertain the state of the liver as well as different types of liver damage (Yakubu *et al*., 2003). Also urea, uric acid creatinine, and serum electrolytes can be used in addition to marker enzymes in diagnosing renal diseases. Assessments of these biochemical parameters offer information about the mechanism of toxicological feat and the functional position of the major organs (Dzoyem *et al.,* 2014). Although enzymes are commonly used to access cellular damage, the choice of enzymes for a particular toxicity study matters because of their compartmentalization. Transaminases (ALT and AST) may be used to access hepatocellular leakages but AST/ALT ratio is more appropriate as it dictate whether the liver or other organ has been damaged since AST is not as specific as ALT (Dzoyem *et al.,* 2014). Alkaline phosphatase, a membrane bound enzyme, has been used as a marker of hepatobiliary and cholestasis (Ramaiah, 2007; Singh *et al.,* 2011). Elevated levels of these enzymes has been linked to tissue damages in diabetes (Ibrahim *et al.,* 2018).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Material

3.1.1 Plant Material and Authentication

Chrysophyllum albidum was obtained in the month of March, 2016, from Ganmo in Ifelodun Local Government Area of Kwara State. Identification and authentication were done at the Herbarium of the Department of Plant Biology, University of Ilorin, Nigeria, where voucher number UILH/001/1170 was assigned and a voucher specimen deposited.

3.1.2 Experimental Animals

Three hundred and forty albino rats (*Rattus norvegicus*) weighing 180.60 ± 8.50 g were obtained from the Animal House of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria.

3.1.3 Drug, Glucometer, Automated Hematological Analyzer and Assay Kits

Glibenclamide used was a product of Medico Remedies Pvt Ltd Juhu, Mumbai, India, while assay kits for glucose-6-phospate dehydrogenase, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, total cholesterol, triglycerides, urea, uric acid, creatinine, albumin, bilirubin were products of Randox Laboratories Ltd., Antrim, UK. C-reactive protein is a product of Bioway Chemistry Reagent Series, Jiang Ning District, Nanjing, China while glycosylated Hb was a product of Fortress Diagnostics Limited, Unit 2C Antrim Technology Park, Antrim, United Kingdom. Alloxan was made by Sigma Chemical Company, St. Louis, Mo, United State of America. Other chemicals used were of analytical grade (JHD, China). Accu-check active glucometer and strips were products of Roche Diagnostic, Mannheim, Germany. Automated hematological analyzer SYSMEX KX21 was a product of SYSMEX Corporation, Harrier, Japan.

3.2 Methods

3.2.1 Preparation of Aqueous Extract of *C. albidum* Stem Bark

Dried stem bark of *C. albidum* (2000g) was cut into smaller pieces and extracted in distilled water (5L) for 72 hours at room temperature. It was sifted using Whatman no 1 filter paper. The filtrate was then freeze-dried (Labconco Freeze Drier, Model 64132, Kansas City, Missouri, USA) to give a percentage yield of 3.76% (75.2g). The extract was reconstituted in distilled water to give the desired doses (25, 50, 100, 200 mg/kg body weight) used for the study.

3.2.2 Ethical Clearance

This study was carried out after ethical approval from the University of Ilorin Ethical Review Committee with approval number UERC/ASN/2017/907 (Appendix IV).

3.3 Induction of Diabetes Mellitus and *In vivo* Antidiabetic Study of Aqueous Extract of *C. albidum* Stem Bark

After overnight fast, the blood glucose levels of the experimental rats were determined before single intra-peritoneal administration of freshly prepared alloxan monohydrate (140 mg/kg body weight) into the rats (Aruna *et al*., 1999). After 48 hours of alloxan injection fasting blood glucose level (FBG) was determined by the means of AccuChek active glucometer and compatible strips. Also, were orally given 5% dextrose saline to overcome the initial hypoglycaemic phase.

3.4 Animal Grouping and Extract Administration

Rats with FBG level above 200 mg/dl were considered diabetic and used for this study. **S**eventy animals were randomly selected into seven groups of ten animals each as follows:

A: Non-diabetic rats +1ml of distilled water

B: Diabetic rats + 1ml of distilled water

C: Diabetic rats + 2.5mg/kg b.wt of glibenclamide

D: Diabetic rats + 25mg/kg b.wt of *C. albidum*

E: Diabetic rats + 50mg/kg b.wt of *C. albidum*

F: Diabetic rats + 100mg/kg b.wt of *C. albidum*

G: Diabetic rats + 200mg/kg b.wt of *C. albidum;*

3.5 Glycemic Studies

Fasting blood glucose (FBG) concentration of all the experimental animals was determined using a glucose oxidase-based commercial glucometer (AccuChek active, Roche Diagnostic) on days (0, 1, 4, 7, 10 and 14) for which the experiment lasted. This was done by drawing blood samples from the sharply cut rats' caudal vein and placing it on the test strip that had been inserted into the glucometer as described by Yakubu and Ogunro (2014).

3.6 Collection of Blood, Preparation of Serum and Tissue Supernatant

The procedure described by Yakubu *et al* (2008) was adopted. Under diethyl ether anesthesia, the animals were sacrificed by simply cutting the jugular vein; the blood was collected into heparinized and plain sample tubes for hematological and serum analysis respectively. Blood samples in the plain sample bottles were left undisturbed at 25 ^{o}C for 30 minutes to form clot after which the samples were centrifuged at 1282 x *g* for 5 minutes. After centrifugation, the supernatant which was the serum was collected by a means of Pasteur pipette into clean sample bottles. The sera, thus obtained were appropriately labeled and stored in freezer at 4 ^{o}C not later than 72 hours of preparation for the biochemical assays. The kidney, liver and pancreas were also removed, freed from fat, blotted with tissue paper and weighed for the computation of organ-body weight ratio. The organs were separately homogenized in ice-cold 0.1M tris buffer (1:5 w/v) using Uniscope Laboratory Centrifuge

(Model SM800B, Surgifriend Medicals, Essex, England). The homogenates were centrifuged at 1789 x *g* for 10 min to obtain the supernatants which were stored frozen and used for biochemical analysis within 72 hours of preparation.

3.7 Determination of Biochemical Parameters

3.7.1 Total Protein

Total protein concentration of the supernatant and serum were determined using the Biuret reagent (Plummer, 1978).

Principle: Copper ions in alkaline medium reacts with peptide bonds in proteins to form a purple coloured complex. The purple colouration is due to the coordination between the cupric ions and the unshared electron pair of peptide nitrogen and the oxygen of water. The colour intensity is a measure of protein content in the sample.

Procedure: 1.0 ml of the sample (appropriately diluted) was added to 4 ml of Biuret's reagent and mixed carefully. The mixture was then left for thirty minutes for colour development at 25 ^{o}C. The corresponding blank was made of 4.0 ml of Biuret's reagent and 1ml of distilled water. The absorbance of the samples was read against blank at 540nm. The bovine serum albumin (BSA) standard curve was obtained by using varying concentrations of BSA (1-10mg/ml).

Calculation*:* Concentration of protein present in the sample was calculated by extrapolating from the BSA standard protein curve on appendix III.

3.7.2 Hepatic Glucose

This was determined using dinitrosalicylic (DNS) reagent (Bernfield, 1955).

Principle: The concentration of 3-amino-5-nitro salicylic acid formed from the reaction of DNS with the carbonyl group of glucose was monitored using spectrophotometry.

Procedure: 0.1 ml of supernatant was diluted with 1 ml of saline solution and mixed with 0.5 ml of DNS reagent. The mixture was incubated in a boiling water bath for 5 minutes and

allowed to cool. The reaction mixture was then diluted by adding 5 ml of distilled water and absorbance was read at 540 nm.

Calculation: Concentration of glucose in each sample was quantified by extrapolating from the standard curve of glucose on appendix III.

3.7.3 Hepatic Glycogen

Glycogen concentration in the liver was determined as glucosyl units following acid hydrolysis (Passoneau and Lauderdale, 1974).

Procedure: Supernatant (0.1 ml) from the homogenized liver was hydrolyzed with 0.5 ml of 1M HCl for 2 hours at 95 ^{o}C. The reaction mixture was neutralized with 0.5 ml of 1M NaOH. Glucose concentrations of the hydrolyzed samples were determined using DNS reagent as previously described.

Calculation: Hepatic glycogen concentration in mg glucose / ml was then calculated as the difference in glucose concentration of the hydrolyzed sample and the non-hydrolyzed (whole) sample.

3.7.4 Hepatic Glucokinase Activity

Hepatic Glucokinase Activity was assayed for using the method of Brandstrup *et al* (1957).

Principle:

Procedure: The reaction mixture contained 1 ml of glucose (0.005 M) solution, 0.5 ml of adenosine triphosphate (0.072 M) solution, 0.1 ml of magnesium chloride (0.05 M) solution, 0.4 ml of potassium dihydrogen phosphate (0.0125 M), 0.4 ml of potassium chloride (0.1 M), 0.4 ml of sodium fluoride (0.5 M) and 2.5 ml of Tris-HCl buffer (0.01 M, pH 8.0). The mixture was then incubated at 37 ^{o}C for 5 minutes. The reaction was initiated by adding 2 ml of tissue supernatant. One millilitre of the reaction mixture was immediately transferred to the tubes containing 1 ml of 10% trichloroethanoic acid that was considered as zero time. A

second aliquot was removed and deproteinised after 30 minutes of incubation at 37 ^{o}C. The protein precipitate was removed by centrifugation at 3000g for 10 minutes and the residual glucose in the supernatant was estimated using the method of Bernfield (1955).

Calculation: Hepatic Glucokinase Activity was calculated by extrapolating from the standard curve of glucose on appendix III.

3.7.5 Glucose -6-phosphate Dehydrogenase (G6PDH) Activity

G6PDH activity was determined as described by Nolltmann *et al* (1961)

Principle: Nictotinamide adenine dinucleotide phosphate (NADP) is reduced by G6PDH in the presence of glucose-6-phosphate. The rate of formation of NADPH is proportional to the G6PDH activity and is measured spectrophotometrically as increase in absorbance at 340nm.

Procedure: Reagent R1, 1.00 ml, 0.03 ml of reagent R2 and 0.015 ml of tissue homogenate was added together in a test tube. The solution was allowed to mix and incubated for 5 minute at 37^{o}C before adding 0.015 ml of reagent R3 and the absorbance read at 340 nm.

Calculation

The enzyme activity was calculated using the following expression:

Enzyme Activity (nmol/min/ml)

$$= \frac{(A_{340}\text{ nm/min Sample} - A_{340}\text{ nm/min blank}) \times 1000 \times V \times df}{6.22 \times V}$$

6.22- Millimolar extinction coefficient of β- NADP at 340 nm

df: Dilution factor

V: Total volume (ml) of the reaction mixture

V: Volume (ml) of sample

1000: The factor introduced to enable enzyme activity is expressed in nmol/min/ml.

The specific enzyme activity (nmol/min/mg protein) = Enzyme Activity/Total Protein Concentration

3.7.6 Alpha Amylase Activity

The method described by Oboh *et al.* (2010) was embraced for the determination of α-amylase.

Procedure: 0.5 ml of liver homogenate was mixed with 0.5 ml of 1 % starch solution prepared in phosphate buffer. The reaction mixtures was incubated at 37 ^{o}C for 10 minutes and stopped with 0.5 ml of DNS reagent. The mixture was then incubated in a boiling water bath for 5 minutes and allowed to cool. The reaction mixture was then diluted by adding 5 ml of distilled water and the absorbance read at 540 nm.

Calculation: the amount of glucose liberated by alpha amylase was calculated by deducing from the standard curve of glucose on appendix III.

3.7.7 Serum Urea Concentration

Serum urea was determined as described by Fawcett and Scott (1960).

$$Urea + H_2O \xrightarrow{urease} 2NH_3 + CO_2$$

Principle: When urea is heated in strongly acidic condition with substance such as diacetyl ($CH_3COCOCH_3COC{=}NaOH$), condensed yellow compounds are formed; the reaction is intensified by the presence of polyvalent ions such as ferric ions which lead to the formation of red coloured complex. The concentration of coloured complex is relative to the concentration of urea in the sample and is measured spectrophotometrically.

Procedure: serum urea concentration was determined by adding 10 µl of the diluted sample to 100 µl of reagent 1 (116 mmol EDTA, 6 mmol/l sodium nitropruside, 1g/l urease). The standard and blank was constituted by adding 10 µl of the standard solution and 10 µl of distilled water to 100 µl of reagent 1 respectively. The mixture was incubated for 10 minutes at 37^{o}C after which 2.5 ml of reagents 2 (120mmol/l phenol) and 3 (27 mmol/l sodium hypochlorate) were added to the solutions. The three solutions were mixed and incubated for

15 minutes at 37^0C. The absorbance was spectrophotometrically read at 550 nm against the reagent blank.

Calculation:

Concentration of urea = $\frac{\text{Absorbance of sample x concentration of standard}}{\text{Absorbance of standard}}$

Concentration of standard (mg/dl) = 78.73

3.7.8 Serum Creatinine Concentration

Serum creatinine concentration was determined using the method Bartels *et al* (1972).

Principle: creatinine in alkaline solution interacts with picric acid to form a coloured complex. The amount of the complex formed is directly related to the amount of creatinine in the sample which can be measured spectrophotometrically.

Procedure: serum creatinine concentration was determined by adding 0.1ml of the sample to 1.0ml of the working reagent. The standard and the blank were reconstituted by adding 0.1ml of the standard solution and 0.1ml of distilled water respectively. The solutions were mixed and 30 seconds later, the absorbance A_1 of the standard and samples were read. After 2 minutes, the absorbance A_2 of the standard and samples were also read at 492 nm.

Calculation*:*

$A_2 - A_1 = \Delta A$ Sample or ΔA Standard

Concentration of creatinine (mg/dl) = $\frac{\Delta A \text{ sample x concentration of standard}}{\Delta A \text{ standard}}$

Concentration of standard (mg/dl) = 2.03

3.7.9 Serum Albumin

Serum albumin concentration was quantified by the method described by Doumas *et al* (1971).

Principle: The determination of serum albumin is based on its quantitative binding to the indicator 3, 3', 5, 5'-tetrabromo-cresol sulphonaptalien (bromocresol green, BCG). The albumin-BCG complex absorbs maximally at 578 nm, the absorbance being directly proportional to the albumin concentration in the sample.

Procedure: To 0.01ml of sample was added to 3.0ml of reagent 1 (75mmol/l of succinate buffer and 0.15mmol/l of bromocresol green). The standard was constituted by replacing the sample with 0.01ml of standard. The blank was constituted by replacing the sample with 0.01ml of distilled water. The mixture was incubated at 37^{o}C for 10 minutes. The absorbance was then read spectrophotometrically at 630nm.

Calculation:

$$\text{Albumin concentration (g/L)} = \frac{\text{Absorbance}_{\text{sample}} \times \text{concentration of standard}}{\text{Absorbance}_{\text{standard}}}$$

Concentration of standard = 5.03 g/L

3.7.10 Determination of Serum Electrolytes

3.7.10.1 Serum sodium ion

Serum sodium ion concentration was determined as described by Tietz (1995).

Principle:

The instrument (flame photometer) used for this assay works on the principle of energy level. The light energy emanating from the gas source is taken by the sodium in the solution and is transmitted to a higher energy level before returning to the ground state. The greater the number of sodium ion the higher the value of sodium.

Procedure:

Appropriately diluted sample (100 µl) was added to 5 ml of working standard. The blank was set up by substituting the sample with the distilled water. The preparation was thoroughly mixed, the galvanometer was put on and the sodium light filter inserted. The gas and air

supply was attuned to obtain discrete blue flame. The blank was used to zero the galvanometer and the sample readings were taken at 590 nm.

Calculation*:*

Sodium (mmol/L) = Galvanometer reading × 2

3.7.10.2 Serum potassium ion

Serum potassium ion concentration was determined as described by Tietz (1995).

Principle:

The flame photometer used for this assay runs on the principle of energy level. The potassium ion in the solution separates to give neutral atoms in the ground state when carefully extracted into a burner. Some of these atoms become agitated in the flame and move to the higher energy level. This light then passes through a suitable filter into a photo-sensitive element. The amount of the current produced is proportional to the amount of potassium in the solution.

Procedure:

Appropriately diluted sample (100 µl) was added to 2500 µl of working standard. The blank was set up by substituting the sample with the distilled water. The preparation was thoroughly mixed, the galvanometer was switched on and the potassium light filter implanted. The gas and air source was attuned to obtain discrete blue flame. The blank was used to zero the galvanometer and adjusted to 4.4 with the working standard. The absorbance of the sample was read against the blank at 770 nm.

Calculation*:*

Potassium ion (mmol/L) = Galvanometer reading

3.7.10.3 Serum chloride ion

Serum potassium ion concentration was determined as described by Tietz (1995).

Principle:

The chloride ions in the sample interact with mercuric thiocyanate to dislodge thiocyanate ion which in turn react with ferric ion to yield a coloured complex that absorbs at 436 nm. The absorbance is proportional to the amount of the chloride ion in the sample.

Procedure:

Appropriately diluted sample (100 µl) was added to a clean tube. Blank and standard were prepared by substituting the sample with 100 µl of distilled water and standard respectively. Working reagent (1 ml) was added to the tubes and the mixture incubated at 37^{o}C for 45 minutes. The absorbance of the test samples and standard were read at 436 against reagent blank.

Calculation*:*

$$\text{Chloride ion concentration (mmol/L)} = \frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

3.7.10.4 Serum bicarbonate ion

Serum bicarbonate ion concentration was determined as described by Tietz (1995).

Principle:

This assay is based on the reaction of phosphoenol pyruvate and bicarbonate in the presence of phosphoenol pyruvate carboxylase to form oxalate and phosphate ion. The oxalate thus made is reduced to male with simultaneous oxidation of NADH to NAD catalyzed by malate dehydrogenase. The reduction in absorbance obtained at 340 nm is proportional to the amount of bicarbonate (CO_2) present in the sample.

Procedure:

One milliliter of working reagent was added to a clean set of tubes labeled sample, standard and blank. The sample, standard and blank was made by adding 0.005 ml of serum, standard

solution and distilled water respectively. The mixture was incubated at 37 ^{o}C for 5 minutes and the absorbance of the sample and standard read at 340 nm against regent blank.

Calculation:

$$\text{Bicarbonate ion concentration (mmol/L)} = \frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

3.7.10.5 Serum phosphate ion

The colorimetric method described by Fiske and Subarrow (1925) was adopted for the determination of phosphate ion in the serum.

Principle:

The principle is based on the reaction of phosphate with acid ammonium molybdate of phosphomolybdic acid to yield phosphomolybdate that further react with a reducing agent (malachite green) to form a green colour. The amount of the colour formed is proportional to the concentration of the phosphate in the sample which is measured spectrophotometrically at 620 nm.

Procedure:

To a clean set of tubes labeled sample, standard and blank, 2.5 ml of working reagent was added. The sample, standard and blank was set up by adding 0.06 ml of serum, standard solution and distilled water respectively. The mixture was left uninterrupted at 25 ^{o}C for 8 minutes and the absorbance of the sample and standard read at 620 nm against regent blank.

Calculation:

$$\text{Phosphate ion concentration (mmol/L)} = \frac{\text{Absorbance of sample} \times \text{concentration of standard}}{\text{Absorbance of standard}}$$

3.8 Determination of Haematological Parameters

Haematological parameters such as red blood cell, haemoglobin, packed cell volume were determined using Sysmex Automated haematology analyzer (Sysmex KX21, Sysme Corporation, Japan) by adopting the procedure described by Dacie and Lewis (1995).

Procedure: the red cells were tallied and lysed by the analyzer releasing the haemoglobin and projected its concentration photometrically. The machine assumed that all the nucleated cells are white blood cells and count them into their different forms (lymphocytes and neutrophils).

3.9 Histological Examination

The method of Drury and Wallington (1980) was employed. Briefly, pancreas specimens were fixed in 10% formalin and dehydrated in ascending grades of ethanol (70%, 90% and 95%), cleared in xylene, and embedded in paraffin wax (melting point 56 ^{o}C) (Krause, 2001; Avwioro, 2010). The tissues were then sectioned (5 µm thick) and stained with Hematoxylin and Eosin stain (H&E). The histology slides were examined using acuscope® (China) microscope with a TSView® Software (China) to observe the pathological changes. Cross section of the liver and kidney were captured at ×400 while those of the pancreas were captured at ×100 with Canon Image Folio package software (Model: Powershot A2500, Japan).

3.10 *In vitro* and *in vivo* Bioactivity Guided Fractionation of Aqueous Extract of *Chrysophyllum albidum* Stem Bark

3.10.1 Solvent-partitioning of Aqueous Extract of *Chrysophyllum albidum* Stem Bark

The procedure of Muhit *et al* (2010) was adopted for the solvent-partitioning of aqueous extract of *C. albidum* stem bark. Briefly, 20 g of the lyophililized aqueous extract was dissolved in 10 ml distilled water and then partitioned in 200 ml of ethyl acetate, and placed on electronic shaker for 45 minutes after which it was poured into a standing

separating funnel and allowed to separate into two distinct phases- the supernatant which is the ethyl acetate-partitioned fraction, and the sediment. The procedure above was repeated before the sediment was extracted in n-butanol. The resulting solvent-partitioned fractions were evaporated to dryness using rotary evaporator to give ethyl acetate-partitioned fraction, n-butanol partitioned fraction and the residue. The fractions and the residue were kept in a desiccator, over silica gel, to ensure complete elimination of water before being used for *in vitro* and *in vivo* studies.

3.10.2 α-Glucosidase Inhibition Assay

The α-glucosidase assay was performed using the method described by Apostolidis *et al* (2006). Aqueous extract of *C.* albidum stem bark and its partitioned fractions (500 µl) and 1000 µl of 0.1 M potassium phosphate buffer (pH 6.90) containing α-glucosidase solution (1.0 U/ml) were incubated in water bath at 25 °C for 10 minutes. After 10 minutes of incubation, 500 µl of 5 mM *p*-nitrophenyl-α-D-glucopyranoside solution in 0.1 M potassium phosphate buffer (pH 6.90) was then added to each tube at 5 minutes interval. The reaction mixture was incubated at 25 °C for 5 minutes prior to the reading of absorbance at 405 nm. The reading was compared with a control which had 500 µl of buffer solution instead of the extract. The α-glucosidase inhibitory activity was expressed as percentage inhibition as follows:

$$\text{Percentage Inhibition} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{extract}}}{\text{Absorbance}_{\text{control}}} \times 100$$

3.10.3 α-amylase Inhibition Assay

This assay was carried out using the procedure of McCue and Shetty (2004). A total of 250 µL of aqueous extract of *C.* albidum stem bark and its partitioned fractions (1.25 – 10 mg/ml) were placed in a tube and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (0.5 mg/ml) was added. The content of the tubes was

incubated at 25 ^{o}C for 10 minutes, after which 250 µL of 1 % starch solution in 0.02 M sodium phosphate buffer (pH 6.9) was added at 5 minutes interval. The reaction mixture was incubated at 25 °C for 10 minutes. The reaction was terminated after the addition of 500 µL of dinitrosalicylic acid (DNS) reagent. The mixture was further incubated in boiling water for 5 minutes and cooled to room temperature. The content of each test tube was diluted with 5 ml of distilled water and the absorbance read at 540 nm using a spectrophotometer (Spectrumlab S23A, Globe Medical, England). A control was prepared using the same procedure except that the extract was replaced with distilled water. The α-amylase activity was calculated using the following expression:

% Inhibition = {(Ac – Ae)/Ac}100 .....(1)

Where Ac and Ae are the absorbance of the control and extract respectively.

3.10.4 Determination of Free Radical Scavenging Activity

The antioxidant activity of the extract and its fractions was determined using the 1, 1-diphenyl-2-picrylhydrazyl **(**DPPH) assay model as described by Yassa *et al* (2008). The extract (50, 100, 250 and 500) µg/ml was prepared in methanol. Each test tube contained 1 ml of the samples and 2 ml of freshly prepared DPPH (40 µg/ml in methanol) solution. Negative control tubes was the same as the test tubes, except that they did not contain DPPH. Absorbance of the mixtures was recorded at 517 nm after 30 minutes, against the blank covets of DPPH solution. Vitamin E was used as a positive control. All samples were assayed in triplicate and IC_{50} value was calculated using the following expression:

Scavenging activity (%) = 1 ($Abs_{sample}/Abs_{control}$) x 100

3.10.5 Determination of Total Antioxidant Capacity (TAC)

The total antioxidant capacity was evaluated using the phosphomolybdenum assay model (Prieto *et al.*, 1999). Briefly, 0.1 mL of extracts was mixed with 1 mL reagent solution (0.6 M Tetraoxosulphate (VI) acid, 28 mM sodium phosphate and 4 mM ammonium

molybdate). The mixture was incubated at 95°C for 90 minutes and allowed to cool. The absorbance of the samples was spectrophotometrically measured at 695 nm against blank. The blank was prepared by replacing the extract with methanol. Total antioxidant capacity was expressed as equivalents of a-tocopherol.

3.11 Glycaemic Study of Aqueous Extract of *C. albidum* Stem Bark and its Fractions

3.11.1 Induction of Diabetes

This was done as earlier described in section 3.3

3.11.1 Animal Grouping and Extract Administration

In order to compare the aqueous and solvent partitioned extracts, seventy animals were randomly selected into seven groups of ten animals each as follows:

A: Non-diabetic rats +1ml of distilled water

B: Diabetic rats + 1ml of distilled water

C: Diabetic rats + 2.5mg/kg b.w of glibenclamide

H: Diabetic rats + 25mg/kg b.wt of ethyl acetate-partition fraction of *C. albidum* stem bark

I: Diabetic rats + 25mg/kg b.wt of residue of crude extract of *C. albidum* stem bark

J: Diabetic rats + 25mg/kg b.wt of n-butanol-partition fraction of *C. albidum* stem bark

K: Diabetic rats + 25mg/kg b.wt aqueous extract of *C. albidum* stem bark

3.12 Anti-diabetic Study of Ethyl Acetate-partitioned Fraction from Aqueous Extract of *C. albidum* Stem Bark

3.12.1 Induction of Diabetes

This was done as earlier described in Section 3.3

3.12.2 Animal Grouping and Extract Administration

For ethyl acetate-partition fraction (best fraction), seventy animals were randomly selected into seven groups of ten animals each as follows:

A: Non-diabetic rats +1ml of distilled water

B: Diabetic rats + 1ml of distilled water

C: Diabetic rats + 2.5mg/kg b.wt of glibenclamide

L: Diabetic rats + 2.5mg/kg b.wt of ethyl acetate-partition fraction of *C. albidum* stem bark

M Diabetic rats + 5mg/kg b.wt of ethyl acetate-partition fraction of *C. albidum* stem bark

N: Diabetic rats + 10mg/kg b.wt of ethyl acetate-partition fraction of *C. albidum* stem bark

O: Diabetic rats + 20mg/kg b.wt of ethyl acetate-partition fraction of *C. albidum* stem bark

3.12.3 Glycaemic Study of Ethyl Acetate-partitioned Fraction from Aqueous Extract of *C. albidum* Stem Bark

This was done as earlier described in Section 3.5

3.12.4 Determination of Hepatic Glucose of Ethyl Acetate-partitioned Fraction from Aqueous Extract of *C. albidum* Stem Bark

This was done as earlier described in Section 3.7.2

3.12.5 Determination of Hepatic Glycogen of Ethyl Acetate-partitioned Fraction from Aqueous Extract of *C. albidum* Stem Bark

This was done as earlier described in Section 3.7.3

3.12.6 Determination of Alpha Amylase Activity of Ethyl Acetate-partitioned Fraction from Aqueous Extract of *C. albidum* Stem Bark

This was done as earlier described in Section 3.7.6

3.12.7 Determination of Glucose-6-phosphate Dehydrogenase Activity of Ethyl Acetate-partitioned Fraction from Aqueous Extract of *C. albidum* Stem Bark

This was done as earlier described in Section 3.7.5

3.12.8 Determination of Serum Lipid profile of Ethyl Acetate-partitioned Fraction from Aqueous Extract of *C. albidum* Stem Bark

3.12.8.1 Determination of Serum Total Cholesterol of Ethyl Acetate-partitioned Fraction from Aqueous Extract of *C. albidum* Stem Bark

The total cholesterol content of the sample was determined after enzymatic hydrolysis and oxidation as described by Roeschlau *et al* (1974).

Principle: The principle is based on the formation of an indicator, quinoneimine from the reaction of hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase as illustrated by the following equations:

Cholesterol ester + H_2O $\xrightarrow{\text{cholesterol esterase}}$ Cholesterol + Fatty acids

Cholseterol + O_2 $\xrightarrow{\text{cholesterol oxidase}}$ Cholestene-3-one + H_2O_2

$2H_2O_2$ + phenol + 4- aminoantipyrine $\xrightarrow{\text{peroxidase}}$ quinoneimine + $4H_2O$

Procedure: the assay mixture consisted of 10 µl each of sample, standard and distilled water (blank) to which 1000 µl of the working reagent (Phosphotungstic Acid and Magnesium Chloride) was added and incubated for 10 minutes at room temperature. The absorbance of the standard and samples was read against reagent blank at 500 nm.

***Calculation**:* Concentration of total cholesterol in the samples

$$= \frac{\Delta A_{\text{sample}} \times \text{Concentration of standard (mg/dl)}}{\Delta A_{\text{standard}}}$$

Where:

ΔA= change in absrbance

3.12.8.2 Determination of Serum Triglycerides of Ethyl Acetate-partitioned Fraction from Aqueous Extract of *C. albidum* Stem Bark

Triglyceride concentration in the serum was determined enzymatically according to the procedure described by Trinder (1969).

Principle: the principle is based on the formation of a colour complex formed from hydrogen peroxide, 4-aminophenozone and 4-chlorophenol under catalytic influence of peroxidase as illustrated in the equations below:

$$\text{Triglycerides} + H_2O \xrightarrow{\textit{lipases}} \text{glycerol} + \text{fatty acids}$$

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

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow[\textit{Oxidase}]{\textit{Glycerol-3-phosphate}} \text{dihydroacetone} + \text{phosphate} + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{\textit{peroxidase}} \text{quinoeimine} + HCl + 4H_2O$$

Procedure: the assay mixture consisted of 10 µl each of sample, standard and distilled water (blank) to which 1000 µl of the working reagent (R1a and R1b; R1a contained pipes buffer, 4- chloro-phenol and Magnesium while R1b contained 4-aminophenazone, ATP, lipases, glycerol kinase, glycerol-3-phosphate oxidase and Peroxidase) was added and incubated for 10 minutes at 25 ^{o}C and the absorbance of sample and standard read against reagent blank at 500 nm.

Calculation:

$$\text{Concentration of triglycerides (mg/dl)} = \frac{\text{Absorbance}_{\text{sample}} \times \text{concentration of standard}}{\text{Absorbance}_{\text{standard}}}$$

3.12.8.3 Determination of Serum of High Density Lipoprotein-Cholesterol of Ethyl Acetate-partitioned Fraction from Aqueous Extract of *C. albidum* Stem Bark

High density lipoprotein- cholesterol (HDL-C) was determined enzymatically by the procedure described by Lopes-Virella (1977).

Principle: Low density lipoproteins (LDL and VLDL) and chylomicrons fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL (high density lipoproteins) fraction which remains in the supernatant was determined as previously described.

Procedure: the HDL (high density lipoproteins) fraction which remains in the supernatant was determined as previously described in Section 3.2.3.1

Calculation*:*

$$\text{Concentration of HDL-C (mg/dl)} = \frac{\text{Absorbance of sample x concentration of standard}}{\text{Absorbance of standard}}$$

3.13 Determination of Lipid Peroxidation of Ethyl Acetate-partitioned Fraction from Aqueous Extract of *C. albidum* Stem Bark

The rate of lipid peroxidation in serum was assessed by quantifying malondialdehyde levels as described by Varshney and Kale (1990).

Principle: This method is based on the reaction of thiobarbituric acid with malondialdehyde in acidic medium to yield a malondialdehyde- thiobarbituric acid adduct, a pink coloured complex that absorbs maximally at 532 nm.

Procedure: 0.8 ml of Tris-KCl was added to 0.2 ml of the sample followed by the addition of 0.25 ml of TCA and 0.25 ml of TBA. The reaction mixture was then incubated for 45minutes at 80^{o}C and cooled on ice. The resulting pink-coloured reaction mixture was centrifuged at

3000 x g for 15minutes. The absorbance of the clear pink supernatant was then read at 532nm using distilled water as blank.

Calculations:

$$\text{MDA (units/mg protein)} = \frac{\text{Absorbance x volume of mixture}}{E_{532} \text{ x volume of sample x mg protein}}$$

Where E_{532} is molar absorbtivity at 532nm = 1.56×10^{-5}

3.14 Determination of Superoxide Dismutase Activity of Ethyl Acetate-partitioned Fraction from Aqueous Extract of *C. albidum* Stem Bark

The method of Misra and Fridovich (1972) was used to determine superoxide dismutase (SOD) activity in the tissues.

Principle: Superoxide dismutase inhibits the auto oxidation of epinephrine at pH 10.2. Superoxide radical ($O^{\cdot -2}$) generated by xanthine oxidase reaction causes the oxidation of epinephrine to adenochrome. The yield of adenochrome produced increases per $O^{\cdot -2}$ introduced as pH and the concentration of epinephrine increases (Valerino and McCormack, 1971).

Procedure: 100 µL of the diluted sample was added to 1000 µL of 0.05M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer. The reaction was started by the addition of 150 µL of freshly prepared 0.3mM epinephrine to the mixture. The reference cuvette contained 1000 µL buffer, 150 µL of epinephrine and 100 µL of distilled water. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds.

Calculations:

$$\text{Increase in absorbance per minute} = \frac{A_5 - A_0}{2.5}$$

Where A_0 = Absorbance after 30 seconds

A_5 = Absorbance after 150 seconds

$$\% \text{ Inhibition} = \frac{\text{Increase in absorbance for substrate} \times 100}{\text{Absorbance of blank}}$$

3.15 Determination of Catalase Activity of Ethyl Acetate-partitioned Fraction from Aqueous Extract of *C. albidum* Stem Bark

Catalase activity was assayed for using the procedure described by Beers and Sizer (1952).

Principle

This method is based on the absorption of hydrogen peroxide by UV light which can be read between 230-250 nm.

Procedure

Hydrogen peroxide (2.9 ml, 0.036% w/w) was mixed with 0.1ml of the homogenate. A blank containing 3ml of 50 mM (pH 7.0) potassium phosphate buffer was prepared. The time required for the absorbance (A_{240} nm) of the reaction mixture to decrease from 0.45 to 0.40 absorbance units was recorded. The activity and specific activity of catalase was calculated using the expression:

$$\text{Enzyme Activity (nmol/min/mL)} = \frac{3.45 \times 1000 \times df}{\text{Min} \times V}$$

3.45 = correspond to the decomposition of 3.45 micromoles of hydrogen peroxide in a 3.0 ml reaction mixture producing a decrease in the A_{240} nm from 0.45 to 0.40 absorbance units

1000= the factor introduced to enable enzyme activity is expressed in nmol/min/ml.

df = dilution factor

Min = time (minutes) required for the $A_{240\ to}$ decrease from 0.45 to 0.40

V = volume (ml) of the sample used

$$\text{Specific enzyme activity (nmol/min/mg protein)} = \frac{\text{Enzyme Activity}}{\text{Protein Concentration}}$$

3.16 Column Chromatography of Ethyl Acetate-partitioned Fraction

The ethyl acetate partitioned extract was of *C. albidum* stem bark was fractionated by the method described by Patil *et al* (2011) using column chromatography with column size of (50 cm x 5cm) and packed with a silica gel (Mesh 60-200). The extract was successively eluted using 50 ml each of ethylacetate – ethanol mixture in the ratios (0:10; 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2; 9:1 and 10:0). After fractionation, the (eleven) chromatographic fractions were pooled using their Rf values from the TLC analysis of the fractions as described by Kumar *et al.*, (2013). The (seven) pooled fractions were evaluated for antidiabetic properties.

3.17 *In vivo* Antidiabetic Study of Column Chromatographic Fraction Treated Diabetic Rats

3.17.1 Induction of Diabetes

This was done as earlier described in Section 3.3

3.17.2 Animal Grouping and Extract Administration

For antidiabetic study of chromatographic fractions, one hundred animals were randomly selected into 10 groups of ten animals each as follows:

A: Non-diabetic rats +1ml of distilled water

B: Diabetic rats + 1ml of distilled water

C: Diabetic rats + 2.5mg/kg b.wt of glibenclamide

F1: Diabetic rats + 2.5 mg/kg b.wt of ethyl acetate chromatographic fraction 1

F3: Diabetic rats + 2.5 mg/kg b.wt of ethyl acetate chromatographic fraction 3

F4: Diabetic rats + 2.5 mg/kg b.wt of ethyl acetate chromatographic fraction 4

F5: Diabetic rats + 2.5 mg/kg b.wt of ethyl acetate chromatographic fraction 5

F7: Diabetic rats + 2.5 mg/kg b.wt of ethyl acetate chromatographic fraction 7

F9: Diabetic rats + 2.5 mg/kg b.wt of ethyl acetate chromatographic fraction 9

F10: Diabetic rats + 2.5 mg/kg b.wt of ethyl acetate chromatographic fraction 10

3.17.3 Glycaemic Study of Column Chromatographic Fraction Treated Diabetic Rats

This was done as earlier described in Section 3.5

3.17.4 Determination of Body weight of Column Chromatographic Fraction Treated Diabetic Rats

The body weight of the experimental rats was determined as described by Oyedemi *et al* (2011) with little modification. The body weight of the rats was taken on days 0, 1, 4, 7, 10 and 14.

3.17.5 Determination of Feed and Water Intake of Column Chromatographic Fraction Treated Diabetic Rats

This was done using the method described by Oyedemi *et al* (2011) with little modification. Briefly, feed and water intake of the experimental rats were taken on the 5^{th} (week1) and 10^{th} (Week 2) days of the experimental period.

3.17.6 Oral Glucose Tolerant Test of Column Chromatographic Fraction Treated Diabetic Rats

On 12^{th} day of the experiment, oral glucose tolerance test was carried out on the experimental animals as described by Attele *et al* (2002) and Ortiz-Andrade *et al* (2008). Briefly, animals were fasted for 4 hours before they were administered test samples (distilled water, glibenclamide, F5 and F7). Thirty minutes later, 2g/Kg glucose was orally administered to the animals and their FBG levels were checked before (0 minutes) and after (30, 60, 90, 120 minutes) glucose administration as earlier described in Section 3.5.

3.17.7 Determination of Hepatic Glucose of Column Chromatographic Fraction Treated Diabetic Rats

This was carried out as earlier described in Section 3.7.2

3.17.8 Determination of Hepatic Glycogen of Column Chromatographic Fractions Treated Diabetic Rats

This was carried out as earlier described in Section 3.7.3

3.17.9 Determination of Serum Insulin Level of Column Chromatographic Fraction Treated Diabetic Rats

Serum insulin was determined using rat insulin ELISA kits provided by Elabscience Biotechnology Company (Shang *et al.*, 2014; Imam *et al.*, 2017).

Principle

The ELISA kit for insulin (Elabscience Biotechnology Co.,Ltd) uses Sandwich-ELISA principle. The micro ELISA plate provided in the kit has been pre-coated with an antibody specific to INS. Standards or samples are added to the appropriate micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for INS and Avidin-Horseradish Peroxidase (HRP) conjugate is added to each micro plate well successively and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain INS, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color turns yellow. The absorbance is read spectrophotometrically at a wavelength of 450 nm. The absorbance value is proportional to the concentration of INS. The concentration of INS in the samples is calculated by comparing the absorbance of the samples to the standard curve.

Procedure: standard or sample (100 µL) was added to each well and incubated for 90 mintues at 37℃. The liquid of the wells was removed and 100 µL biotinylated detection working solution was added and incubated for 1 hour at 37℃. The wells were aspirated and washed 3 times with wash buffer after which 100 µL HRP conjugate was added and incubated for 30 minutes at 37℃. The liquid of the wells was aspirated, washed 5 times after

which 90 µL of the substrate reagent was added and incubated for 15 minutes at 37℃ before 50 µL of stop solution was added. The absorbance was read at 450nm immediately using micro-plate reader.

3.17.10 Determination of Glucokinase Activity of Column Chromatographic Fraction Treated Diabetic Rats

This was carried out as earlier described in Section 3.7.4

3.17.11 Determination of Glucose-6-Phosphatase Activity of Column Chromatographic Fraction Treated Diabetic Rats

Glucose-6-phosphatase activity was determined following the method described by Koide and Oda (1959).

Principle:

Procedure: Incubation mixture contained 0.7 ml of citrate buffer (0.1 M, pH 6.5), 0.3 ml of substrate (glucose-6-phosphate, 0.01 M) and 0.3 ml of tissue homogenate. The reaction mixture was incubated at 37 ºC for 1 hour. Addition of 1 ml of 10% trichloroethanoic acid to the reaction tubes terminated the reaction of the enzyme. The suspension was centrifuged at 3000g for 5 minutes and the phosphorus content of the supernatant was estimated by the method of Fiske and Subbarow (1925). 0.5 ml of supernatant was added 1 ml of ammonium molybdate and 0.4 ml of amino naphthol sulphonic acid. The blue colour developed after 20 minutes was read at 680 nm.

Calculation: the amount of phosphate liberated by glucose-6-phosphatase was calculated by extrapolating from the standard curve of phosphate on Appendix III.

3.17.12 Determination of Fructose-1,6-bisphosphatase Activity of Column Chromatographic Fraction Treated Diabetic Rats

Fructose-1, 6-bisphosphatase activity was determined using the method described by Gancedo and Gancedo (1971).

Procedure: the assay mixture contained 1.2 ml of Tris-HCl buffer (0.1 M, pH 7.0), 0.1 ml of substrate (fructose-1,6-bisphosphate, 0.05 M), 0.25 ml of magnesium chloride (0.1 M), 0.1 ml of potassium chloride solution (0.1 M), 0.25 ml of ethylenediaminetetraacetic acid (0.001 M) solution and 0.1 ml of supernatant. The incubation was carried out at 37 ^{o}C for 5 minutes. The reaction was terminated by adding of 10% trichloroethanoic acid. The suspension was centrifuged at 3000g for 5 minutes and the supernatant obtained was used for phosphorus estimation as previously described.

Calculation: the amount of phosphate liberated by fructose1,6-bisphosphatase was calculated by extrapolating from the standard curve of phosphate on Appendix III.

3.17.13 Determination of Glucose-6-Phosphate Dehydrogenase Activity of Column Chromatographic Fraction Treated Diabetic Rats

This was carried out as earlier described in Section 3.7.5

3.17.14 Determination of C-Reactive Protein of Column Chromatographic Fraction Treated Diabetic Rats

The method described by Burtis and Ashwood (1999) was used for determination of serum CRP level.

Principle: The kit utilizes latex-enhanced immunoturbidimetry to measure the CRP level in serum. During the test, CRP in the sample binds with the specific anti-CRP antibody that is coated on latex particles to cause agglutination. The turbidity caused by agglutination is detected optically, the absorbance of which is proportional to the level of CRP in the sample.

Procedure: 2 µl of sample was added to 225 µl of R1 (glycine buffer solution, sodium azide), mixed and incubated at 37°C for 4 minutes after which 75 µl of R2 (latex suspension, anti-CRP antibodies, glycine buffer solution, sodium azide), was added, mixed thoroughly and incubated at 37°C for 10 seconds. Absorbance 1 (Ab1) was taken and absorbance 2 (Ab2) was also taken 5 minutes after the first reading.

Calculation: The actual concentration is obtained by comparing with a calibration curve with known concentrations. $\Delta Ab = Ab2 - Ab1$

3.17.15 Determination of Glycosylated Haemoglobin Level of Column Chromatographic Fraction Treated Diabetic Rats

Glycosylated haemoglobin level was determined as described by Trivelli *et al* (1971).

Principle

A haemolysed preparation of the whole blood is mixed continuously for 5 minutes with a weak binding cation-exchange resin. During this time, the non-glycosylated haemoglobin (HbAo) binds to the resin. After the mixing period, a filter is used to separate the supernatant containing the glycohaemoglobin from the resin. The percent glycohaemoglobin is determined by measuring the absorbance of the glycohaemoglobin fraction and the total haemoglobin fraction at 415 nm. The ratio of the two absorbances gives the percent glycohaemoglobin.

Procedure:

For glycol-haemoglobin, 500 µl of lysing reagent was added to tubes containing 100 µl of well-mixed blood sample, standard, and control. The mixture was left for 5 minutes. Thereafter, 100 µl of the haemolysate was added to 3 ml of glycohaemoglobin cation-exchange resin, mix for 5 minutes and the mixture centrifuged at 2000g for 10 minutes after which the supernatant was removed and the absorbance read at 415 nm with deionized water as the blank. To a separate set of tubes, 20 µl of the haemolysate was also added to pre-

labeled tubes containing 5.0 ml deionized water, mixed and the absorbance read at 415nm with deionized water as the blank.

Calculation:

$$\% \text{ Glycosylated Hb (unknown)} = \frac{\text{R (unknown)} \times \text{Standard concentration}}{\text{R (standard)}}$$

Where:

$$\text{R (unknown)} = \text{Ratio (unknown)} = \frac{\text{Abs. of Glycosylated Hb (unknown)}}{\text{Abs. of Total Hb (unknown)}}$$

$$\text{R (standard)} = \text{Ratio (standard)} = \frac{\text{Abs. of Glycosylated Hb (standard)}}{\text{Abs. of Total Hb (standard)}}$$

3.17.16 Determination of Serum Lipid Profile of Column Chromatographic Fraction Treated Diabetic Rats

The total cholesterol, triglycerides and high density lipoprotein-cholesterol was done as previously described in Section 3.11.8 while low density lipoproteins (LDL and VLDL) cholesterol was calculated as per Friedewald's equation (Friedewald *et al.,* 1972).

VLDL-C = 1/5 of Triglyceride; and

LDL-C = Total cholesterol – (VLDL-C + HDL-C)

Atherogenic Index = (LDL-C/ HDL-C)

3.17.17 Determination of Antioxidant Enzyme Activity and Lipid Peroxidation Level of Column Chromatographic Fraction Treated Diabetic Rats

Determination of superoxide dismutase, catalase and malondialdehyde was done as previously described in Sections 3.13, 3.14 and 3.12 respectively.

3.18 Histological Examination of Column Chromatographic Fraction Treated Diabetic Rats

This was done as earlier described in Section 3.9

3.19 Acute Toxicity Study

3.19.1 Animal Grouping and Extract Administration

For acute toxicity study, thirty animals were randomly selected into three groups of ten animals each as follows:

Control: rats + 1ml of distilled water

F5: rats + 2.5 mg/kg b.wt (1ml) of ethyl acetate chromatographic fraction 5

F7: rats + 2.5 mg/kg b.wt (1ml) of ethyl acetate chromatographic fraction 7

3.19.2 Determination of Some Function Indices

Urea, creatinine, albumin, bilirubin were determined as earlier described in Sections 3.7.7, 3.7.8, 3.7.9 respectively while total and direct bilirubin were determined as described by Sherlock (1951).

Principle: bilirubin in the serum reacts with diazotized sulphanilic acid in alkaline medium (direct) or with caffeine (total) to form blue coloured complex which is measured spectrophotometrically.

Procedure: total bilirubin was determined by adding 50µl of reagent 2 (38.5 mmol/l of sodium nitrite) to 200µl of reagent 1 (29 mmol/l sulphanilic acid) after which 100µl of reagent 3 (0.26 mol/l caffeine) was added. Then 200µl of sample was added to the mixture. The blank was constituted by replacing the sample with distilled water without reagent 2. Conjugated bilirubin was determined by adding 50µl of reagent 2 to 200µl of reagent 1 followed by the addition of 200µl of 0.9% NaCl. The sample (200µl) was then added to the mixture. The blank was constituted by replacing sample with distilled water without adding reagent 2. Both total and conjugated bilirubin was mixed and incubated at 25^{o}C for 10

minutes after which 100µl of reagent 4 (0.93mol/l tartrate) was added to the total bilirubin preparation and the absorbance was read spectrophotometrically at 546nm.

Calculation:

Total bilirubin (mg/dl) = 10.8 x A_{TB}

Direct bilirubin (mg/dl) = 14.4 x A_{DB}

A_{TB} = Absorbance of total billirubin

A_{DB} = Absorbance of direct billirubin

10.8 and 14.4 are total and direct bilirubin concentrations respectfully.

3.19.3 Determination of Serum Electrolytes

Serum electrolytes was determined as described in Section 3.10

3.19.4 Determination of Alkaline Phosphatase Activity

Alkaline phosphatase (ALP) was assayed using the method described by Wright *et al* (1972a) which employs the use of ρ-nitrophenyl phosphate as substrate.

Principle: the amount of phosphate ester split within a given period of time is a measure of the phosphatase enzyme. Para-nitrophenyl phosphate (ρNPP) was hydrolyzed to para-nitrophenol and phosphate. The para-nitrophenol confers a yellowish colour on reaction mixture and its intensity was read spectrophotometrically at 400 nm.

Procedure: 2.2 ml of carbonate buffer (0.1M, pH 10.1) was dispensed into clean test-tube and 0.1 ml of 0.1M MgSO4 and 0.2 ml of the enzyme source (serum or tissue homogenate) was added. The mixture was equilibrated in water bath for 10 minutes. An aliquot (0.5 ml) of 19 mM of ρ-nitrophenyl phosphate was added and the mixture incubated at 37 ^{o}C for 10 minutes. The reaction was stopped with 2.0 ml of 1N NaOH and the absorbance read at 400 nm. Blank was prepared by replacing enzyme source with distilled water in the test tube labeled blank.

Calculation:

Enzyme activity (nM/min/ml) = $\frac{\text{Absorbance/min x 1000 x TV x F}}{\text{9.9 x SV x d}}$

Where,

Absorbance/min = Absorbance of the reaction mixture per minute

TV = Total volume of reaction mixture

F = dilution factor

SV= Volume of enzyme source (sample)

d = Light path length (1cm)

9.9 = Extinction coefficient of 1 mole of ρ-nitrophenol in alkaline solution of 1ml volume of 1cm light path at 400nm.

1000 = Factor introduced to enable enzyme activity to be expressed in nM/min/ml

Therefore,

Specific activity (nM/min/mg protein) = $\frac{\text{Enzyme activity (nM/min/ml)}}{\text{Protein concentration (mg/ml)}}$

3.19.5 Determination of Aspartate Aminotransferase Activity

The activity of aspartate aminotransaminase (AST) in the serum and tissue homogenates was determined following the method described by Reitman and Frankel (1957).

Principle: the Enzyme catalyzes the reversible reaction involving α-ketoglutarate and L-aspartate to from L-glutamate and Oxloacetate. Aspartate aminotransferase activity was determined by monitoring the concentration of oxaloacetate hydrazones formed with 2,4-dinitrophenyl hydrazine.

Procedure: 0.5 ml of reagent 1 (100mmol/l of Phosphate buffer pH 7.4, 100mmol/l of L-aspartate and 2mmol/l of oxoglutarate) was added to 0. 1ml of sample. The blank was

constituted by replacing the sample with distilled water. The mixture was incubated for 30 minutes at 37ºC after which 0.5 ml of reagent 2 (2mmol/l of 2.4-dinitrophenylhydrazine) was added. The mixtures was left for 20 minutes at 37ºC after which 0.5 ml of 0.4 mol/L NaOH was added to stop the reaction. The solution was mixed and the absorbance read against blank after 5 minutes at 546 nm.

3.19.6 Determination of Alanine Aminotransferase Activity

The activity of alanine transaminase (ALT) in the serum and tissue homogenates was determined following the method reported by Reitman and Frankel (1957).

Principle: Alanine aminotransferase activity was determined by monitoring the reaction of pyruvate hydrazone with 2, 4-dinitrophenyl hydrazine.

Procedure: 0.5 ml of reagent 1 (100mmol/l of Phosphate buffer pH 7.4, 200 mmol/l of L-alanine and 2.0 mmol/l of oxoglutarate) was added to 0.1 ml of the sample. The blank was constituted by replacing the sample with distilled water. The mixture was incubated for 30 minutes at 37ºC after which 0.5 ml of reagent 2 (2.0 mmol/l of 2,4- dinitrophenylhydrazine) was added. The mixture was left for 20 minutes at 25ºC after which 0.5 ml of 0.4mol/L NaOH was used to stop the reaction. The reaction was mixed and absorbance read against blank after 5 minutes at 546 nm.

3.20 High Performance Chromatography-Diode Array Detection (HPLC-DAD) Analysis of Ethyl Acetate-Partitioned Fraction F7

Reverse phase chromatography analysis was carried out under gradient conditions using C18 column (4.6 mm, 7mm, 150 mm; Ubondupak, Nevera mix, Shimadzu). The mobile phase was water containing water (A) and acetonitrile (B), and the composition gradient was: 17% of B until 10 minutes and changed to obtain 20, 30, 50, 60, 70, 20, and 10% B at 20, 30, 40, 50, 60, 70, and 80 minutes, respectively, following the method described by Brito *et al* (2015). The samples (F7 and standards) and mobile phase were filtered through

0.45 µL membrane filter (Millipore), degassed and analysed at a concentration of 15 mg/mL. The flow rate was 1 mL/minute; injection volume was 20 µL. Chromatography peaks were confirmed by comparing retention times with those of reference standards and by DAD spectra (200–500 nm). All chromatography operations were carried out at ambient temperature.

3.21 Data Analysis

Data were expressed as the mean ± SEM of ten determinations except on acute toxicity and *in vitro* studies where sample size were seven and three respectively. Data were analyzed using one way analysis of variance followed by Tukey's *post-hoc* test for multiple comparisons. Statistical significance was set at 95% confidence interval ($p < 0.05$) and graph Pad statistical Package version 6.0 was used for the statistical analyses.

CHAPTER FOUR

RESULTS

4.1 *In vivo* Anti-diabetic Study of Aqueous Extract of *Chrysophyllum albidum* Stem bark in Alloxan-induced Diabetic Rats

4.1.1 Fasting Blood Glucose of Diabetic Rats Orally Administered Aqueous Extract of *Chrysophyllum albidum* Stem Bark

Figure 11 shows fasting blood glucose (FBG) levels of diabetic rats orally administered aqueous extract of *Chrysophyllum albidum* stem bark for 14 days. The FBG levels of the rats administered alloxan significantly ($p < 0.05$) increased from 80.00 to 506.00 mg/dL. Administration of aqueous extract of *Chrysophyllum albidum* stem bark at various doses (25, 50, 100 and 200 mg/Kg body weight) significantly ($p < 0.05$) reduced the FBG level steadily till the end of the experimental period. The reduction in FBG of alloxan-induced diabetic rats given 25 mg/kg body weight of the extract were not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats while the FBG level of alloxan-induced diabetic rats given 50, 100 and 200 mg/kg body weight of the extract were not considerably ($p < 0.05$) different from the glibenclamide-treated diabetic rats.

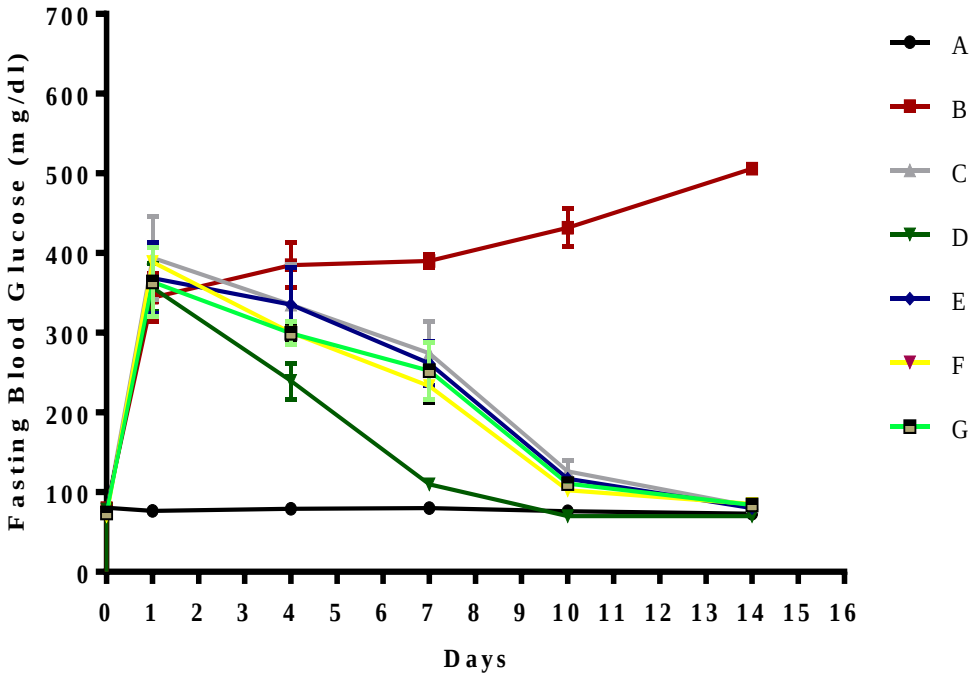


Figure 11: Fasting Blood Glucose of Diabetic Rats orally Administered Aqueous Extract of *Chrysophyllum albidum S*tem Bark

Where:

A = Non diabetic rats + 1mL distilled water

B = Diabetic + 1mL distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

D = Diabetic rats + 25 mg/kg body weight of aqueous extract of *C. albidum* stem bark

E = Diabetic rats + 50 mg/kg body weight of aqueous extract of *C. albidum* stem bark

F = Diabetic rats + 100 mg/kg body weight of aqueous extract of *C. albidum* stem bark

G = Diabetic rats + 200 mg/kg body weight of aqueous extract of *C. albidum* stem bark

4.1.2 Effects of Aqueous Extract of *Chrysophyllum albidum* Stem Bark on Hepatic Glucose and Glycogen Contents of Alloxan-induced Diabetic Rats

Administration of alloxan monohydrate significantly ($p < 0.05$) increased the hepatic glucose with corresponding decrease in glycogen level when compared with distilled water treated non-diabetic rats. Administration of the extracts at 25 mg/kg body weight dose reversed the alloxan-induced increase in hepatic glucose with its corresponding decrease in glycogen level which was not considerably different from the distilled water treated non-diabetic rats. Diabetic rats administered 50, 100 and 200 mg/kg body weight extract were not significantly ($p < 0.05$) different from the distilled water treated diabetic rats.

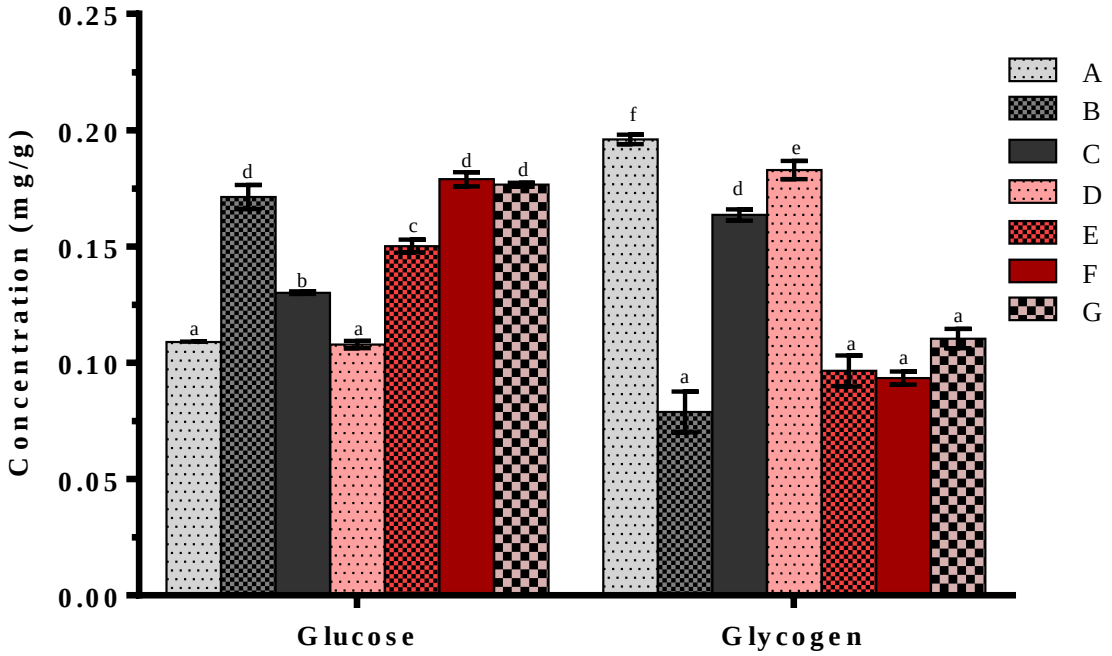


Figure 12: Hepatic Glucose and Glycogen Level of Diabetic Rats orally Administered Aqueous Extract of *Chrysophyllum albidum* Stem Bark

A = Non diabetic rats + 1mL distilled water

B = Diabetic + 1mL distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

D = Diabetic rats + 25 mg/kg body weight of aqueous extract of *C. albidum* stem bark

E = Diabetic rats + 50 mg/kg body weight of aqueous extract of *C. albidum* stem bark

F = Diabetic rats + 100 mg/kg body weight of aqueous extract of *C. albidum* stem bark

G = Diabetic rats + 200 mg/kg body weight of aqueous extract of *C. albidum* stem bark

4.1.3 Effects of Aqueous Extract of *Chrysophyllum albidum* Stem bark on the Activity of Some Carbohydrate Metabolizing Enzymes of Alloxan-induced Diabetic Rats

Compared with the distilled water treated non-diabetic rats, administration of alloxan significantly ($p < 0.05$) decreased the activity of glucokinase (Figure 13). Administration of the extract at 25 and 100 mg/kg body weight doses of the extract significantly increased the activity of the enzyme and and was not significsntly different from the distilled water treated non-diabetic rats while 50 and 200 mg/kg body weight of the extract were not different from the diabetic rats given glibenclamide.

Administration of the extract at all the doses reversed the alloxan-induced significant ($p < 0.05$) decrease in glucose-6-phosphate dehydrogenase activity (Figure 14). The extract at 25 and 50 mg/kg body weight doses were not significantly different from the distilled water treated non-diabetic rats while 100 and 200 mg/kg body weight of the extract were though significantly increased the activity of the enzyme, they did not compared well with neither the distilled water treated non-diabetic rats nor the reference drug treated diabetic rats.

Compared with the distilled water treated non-diabetic rats, α-amylase activity significantly ($p < 0.05$) increased in the small intestine, pancreas and liver (Figure 15) of the distilled water treated diabetic rats. Although, activity of alpha amylase in the tissues significantly ($p < 0.05$) reduced following oral administration of doses of the extract, only 25 mg/kg body weight of the extract were not significantly different from the distilled water treated non-diabetic rats.

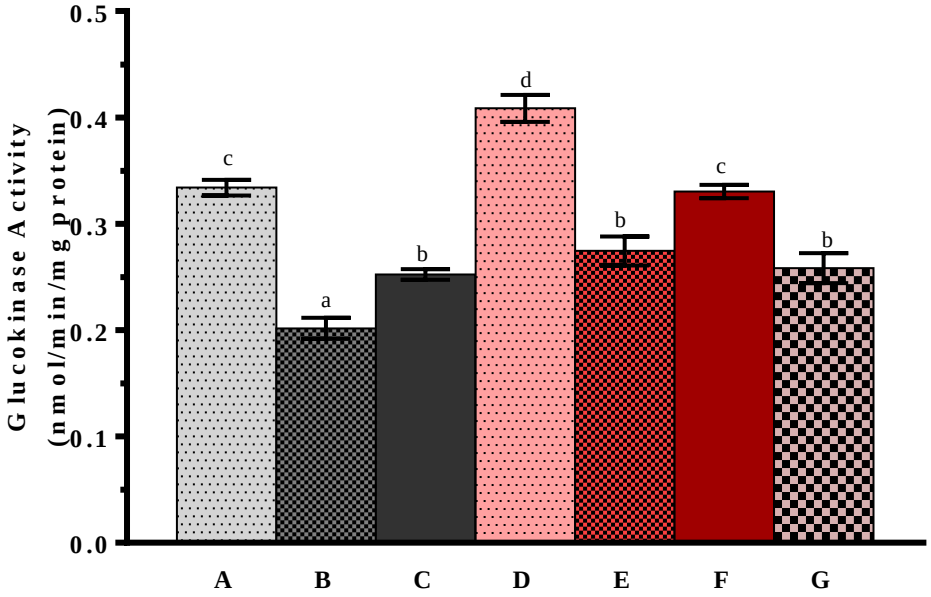


Figure 13: Hepatic Gucokinase Activity of Diabetic Rats Orally Administered Aqueous Extract of *Chrysophyllum albidum* Stem Bark

A = Non diabetic rats + 1mL distilled water

B = Diabetic + 1mL distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

D = Diabetic rats + 25 mg/kg body weight of aqueous extract of *C. albidum* stem bark

E = Diabetic rats + 50 mg/kg body weight of aqueous extract of *C. albidum* stem bark

F = Diabetic rats + 100 mg/kg body weight of aqueous extract of *C. albidum* stem bark

G = Diabetic rats + 200 mg/kg body weight of aqueous extract of *C. albidum* stem bark

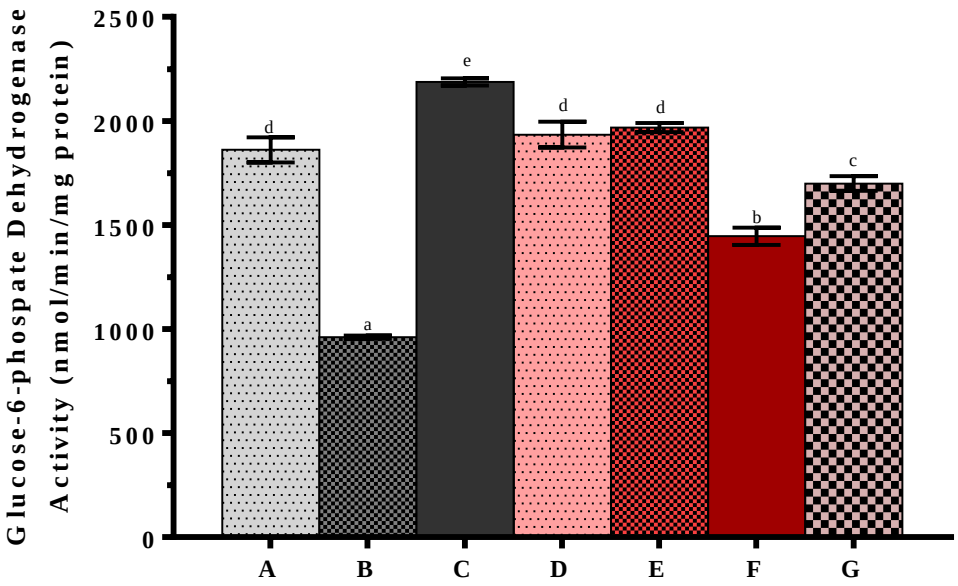


Figure 14: Hepatic glucose -6-phosphate Dehydrogenase Activity of Diabetic Rats Orally Administered Aqueous Extract of *Chrysophyllum albidum* Stem Bark

A = Non diabetic rats + 1mL distilled water

B = Diabetic + 1mL distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

D = Diabetic rats + 25 mg/kg body weight of aqueous extract of *C. albidum* stem bark

E = Diabetic rats + 50 mg/kg body weight of aqueous extract of *C. albidum* stem bark

F = Diabetic rats + 100 mg/kg body weight of aqueous extract of *C. albidum* stem bark

G = Diabetic rats + 200 mg/kg body weight of aqueous extract of *C. albidum* stem bark

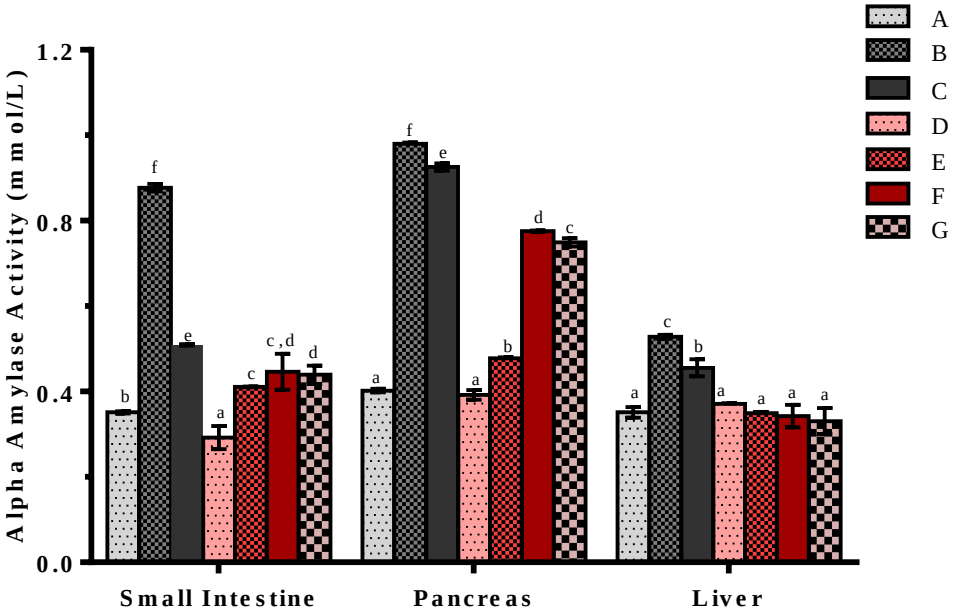


Figure 13: Activity of Alpha Amylase of Diabetic rats Orally Administered Aqueous Extract of *Chrysophyllum albidum* Stem Bark

A = Non diabetic rats + 1mL distilled water

B = Diabetic + 1mL distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

D = Diabetic rats + 25 mg/kg body weight of aqueous extract of *C. albidum* stem bark

E = Diabetic rats + 50 mg/kg body weight of aqueous extract of *C. albidum* stem bark

F = Diabetic rats + 100 mg/kg body weight of aqueous extract of *C. albidum* stem bark

G = Diabetic rats + 200 mg/kg body weight of aqueous extract of *C. albidum* stem bark

4.1.4 Effects of Aqueous Extract of *Chrysophyllum albidum* Stem Bark on Urea and Creatinine Levels of Alloxan-induced Diabetic Rats

Compared with the distilled water treated non-diabetic rats, administration of alloxan significantly ($p < 0.05$) increased the serum urea and creatinine levels (Figure 16). Administration of the extract of all doses of the extract though, significantly ($p < 0.05$) decreased the levels of urea and creatinine but only 25 mg/kg body weight compared well with the distilled water treated non-diabetic rats.

4.1.5 Effects of Aqueous Extract of *Chrysophyllum albidum* Stem Bark on Serum Albumin Level of Alloxan-induced Diabetic Rats

Compared with the distilled water treated non-diabetic rats, administration of alloxan significantly ($p < 0.05$) decreased the serum albumin level (Figure 17). Administration of the extract at all doses of the extract were not significantly ($p < 0.05$) different from distilled water treated non-diabetic rats.

4.1.6 Effects of Aqueous Crude Extract of *Chrysophyllum albidum* Stem Bark on Erythrocytic Indices and Serum Electrolytes of Alloxan-induced Diabetic Rats

Compared with the distilled water treated non-diabetic rats, administration of alloxan significantly ($p < 0.05$) reduced the electrolytes and the erythrocytic indices (RBC, Hb and PCV). Administration of the extract at all doses significantly ($p < 0.05$) increased the erythrocytic indices and were not significantly different ($p < 0.05$) from the glibenclamide treated diabetic rats except the RBC of the 100 and 200 mg/kg body weight treated groups which were not significantly different ($p < 0.05$) from the distilled water treated non-diabetic rats. Also, only the electrolytes of the 25 mg/kg body weight of the diabetic treated group were not significantly different ($p < 0.05$) from the distilled water treated non-diabetic rats (Table 5).

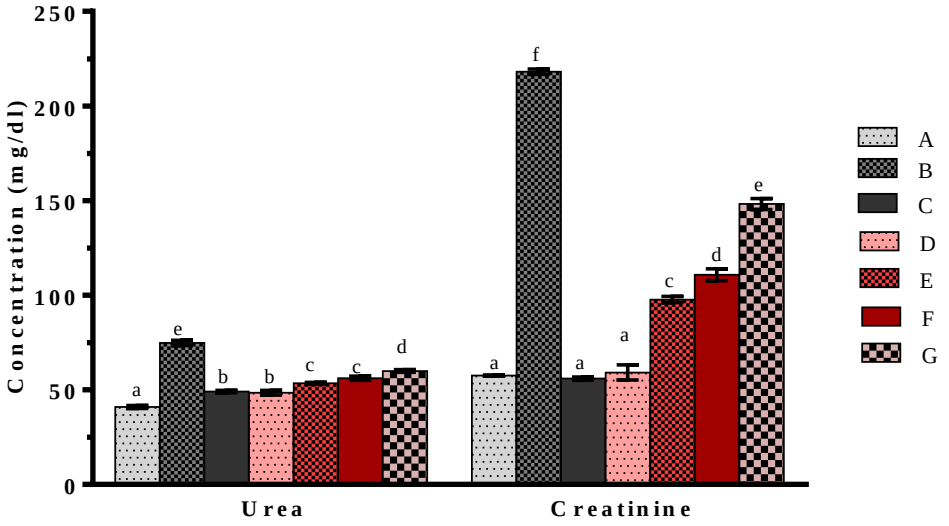


Figure 14: Serum Urea and Creatinine Concentration of Diabetic Rats Orally Administered Aqueous Extract of *Chrysophyllum albidum* Stem Bark

A = Non diabetic rats + 1mL distilled water

B = Diabetic + 1mL distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

D = Diabetic rats + 25 mg/kg body weight of aqueous extract of *C. albidum* stem bark

E = Diabetic rats + 50 mg/kg body weight of aqueous extract of *C. albidum* stem bark

F = Diabetic rats + 100 mg/kg body weight of aqueous extract of *C. albidum* stem bark

G = Diabetic rats + 200 mg/kg body weight of aqueous extract of *C. albidum* stem bark

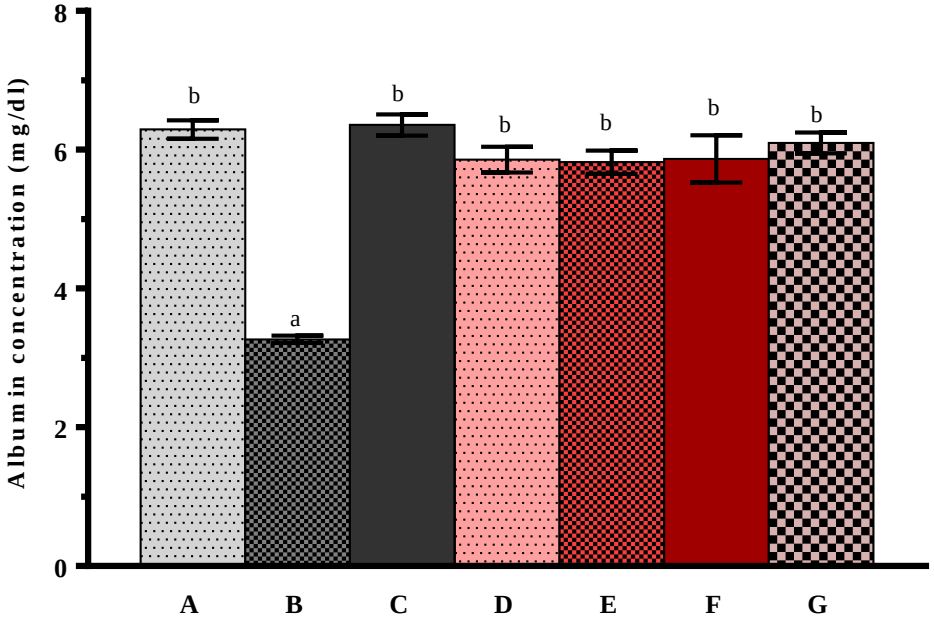


Figure 15: Serum Albumin concentration of diabetic rats orally administered aqueous extract of *Chrysophyllum albidum* stem bark

A = Non diabetic rats + 1mL distilled water

B = Diabetic + 1mL distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

D = Diabetic rats + 25 mg/kg body weight of aqueous extract of *C. albidum* stem bark

E = Diabetic rats + 50 mg/kg body weight of aqueous extract of *C. albidum* stem bark

F = Diabetic rats + 100 mg/kg body weight of aqueous extract of *C. albidum* stem bark

G = Diabetic rats + 200 mg/kg body weight of aqueous extract of *C. albidum* stem bark

Table 5: Erythrocytic Indices and Serum Electrolytes (mmol/L) of Diabetic Rats Orally Administered Aqueous Extract of *Chrysophyllum albidum* Stem Bark

Parameters/ Group	Non diabetic rats + 1mL distilled water	Diabetic + 1mL distilled water	Diabetic rats + 2.5 mg/kg body weight of glibenclamide	Diabetic rats + 25 mg/kg body weight AECASB	Diabetic rats + 50 mg/kg body weight of AECASB	Diabetic rats + 100 mg/kg body weight of AECASB	Diabetic rats + 200 mg/kg body weight of AECASB
RBC ($\times 10^6$)	6.27 ± 0.15^{c}	3.72 ± 0.13^{a}	5.53 ± 0.28^{b}	5.10 ± 0.51^{b}	5.50 ± 0.14^{b}	6.40 ± 0.22^{c}	6.25 ± 0.38^{c}
Hb (g/dl)	11.00 ± 0.10^{c}	5.95 ± 0.25^{a}	9.30 ± 0.50^{b}	8.90 ± 0.10^{b}	9.50 ± 0.20^{b}	10.05 ± 0.55^{b}	9.55 ± 0.35^{b}
PCV (%)	37.30 ± 0.20^{c}	17.40 ± 0.70^{a}	32.15 ± 0.35^{b}	31.25 ± 0.55^{b}	33.05 ± 1.15^{b}	30.95 ± 0.55^{b}	31.70 ± 0.70^{b}
Sodium	14.13 ± 091^{c}	6.38 ± 0.64^{a}	9.21 ± 0.71^{b}	13.84 ± 1.11^{c}	10.01 ± 1.06^{b}	9.34 ± 0.87^{b}	9.44 ± 0.64^{b}
Potassium	6.37 ± 0.24^{e}	2.18 ± 0.22^{a}	4.21 ± 0.16^{c}	5.44 ± 0.11^{d}	3.94 ± 0.26^{b}	3.71 ± 0.27^{b}	4.01 ± 0.06^{c}
Chloride	9.48 ± 1.06^{c}	4.11 ± 0.38^{a}	6.24 ± 0.26^{b}	8.85 ± 0.91^{c}	5.92 ± 0.13^{b}	6.10 ± 0.12^{b}	5.89 ± 0.20^{b}
Carbonate	3.81 ± 0.04^{a}	5.96 ± 0.21^{b}	3.51 ± 0.22^{a}	3.91 ± 0.03^{a}	6.01 ± 0.11^{b}	5.81 ± 0.04^{b}	5.73 ± 1.26^{b}
Phosphate	3.11 ± 0.24^{a}	8.17 ± 0.24^{c}	5.01 ± 0.33^{b}	3.21 ± 0.14^{a}	7.92 ± 0.22^{c}	7.81 ± 1.02^{c}	8.02 ± 0.24^{c}

Values are mean of ten replicates ± SEM and values with different superscripts on the same row are significantly different.

Where AECASB = aqueous extract of *Chrysophyllum albidum* stem bark

4.2 Bioactivity guided Study

4.2.1 Yield of Solvent-partitioned Fractions from Crude Aqueous Extract of *Chrysophyllumalbidum* Stem Bark

The solvent partitioning of aqueous extract of *Chrysophyllum albidum* stem bark produced three fractions: ethyl acetate partitioned fraction (0.8 g), n-butanol partitioned fraction (6.2 g) and residue (12.4 g). The ethyl acetate partitioned fraction had the least percentage yield (4.0%) while the residue had the highest yield (62.0%).

4.2.2 *In vitro* Anti-diabetic Study

Table 6 shows mean inhibitory concentration (IC_{50}) of the aqueous and solvent partition fractions of *Chrysophyllum albidum* stem bark on enzymes linked to carbohydrate metabolism. The aqueous extract shows the highest inhibition against α-amylase while n-butanol partitioned fraction exhibited the least inhibition (Table 6). N-butanol which is not significantly ($p < 0.05$) different from the residue had the highest inhibition against α-glucosidase while ethyl acetate had the least inhibition against α-glucosidase.

4.2.3 *In vitro* DPPH Radical Scavenging Activity and Total Antioxidant Capacity of Aqueous and Solvent-partitioned Fractions of *Chrysophyllum albidum* Stem Bark

Table 7 shows mean effective concentration (EC_{50}) of the total antioxidant capacity (TAC) and DPPH radical scavenging activity of aqueous and solvent-partitioned extracts of *Chrysophyllum albidum.* The ethyl acetate-partitioned fraction displayed the highest EC_{50} among the solvent-partitioned fractions and compared well with the crude. The ethyl acetate-partitioned fraction displayed highest EC_{50} among the fractions while n-butanol and residue where not significantly different from each other.

Table 6: Mean Inhibitory Concentration (IC_{50}) of the Crude and Solvent Partition Extracts of *Chrysophyllum albidum* on Enzymes Linked to Carbohydrate Metabolism

Extract	α-amylase (µg/ml)		α-glucosidase (µg/ml)	
	IC_{50}	r^2	IC_{50}	r^2
Crude	194.55 ± 11.21^{a}	0.9673	49.70 ± 2.88^{bc}	0.9679
Ethyl acetate	292.06 ± 3.60^{c}	0.9684	53.63 ± 2.25^{c}	0.9387
Residue	257.60 ± 4.81^{b}	0.9654	42.05 ± 2.37^{ab}	0.9449
Butanol	327.23 ± 3.20^{d}	0.9780	38.02 ± 2.50^{a}	0.8077

*r^2= regression coefficient

Values are mean of three replicates ± SEM; Values with different superscripts for each enzyme are significantly ($p < 0.05$) different from others

Table 7: Mean effective concentration (EC_{50}) of the DPPH radical scavenging activity and total antioxidant capacity (TAC) of crude and solvent partition extracts of *Chrysophyllum albidum*

Extract	DPPH (mg/ml) EC_{50}	r^2	TAC (mg/ml)	r^2
Crude	2.89 ± 0.24^{c}	0.96	3.16 ± 0.24^{c}	0.96
Ethyl acetate-partitioned	2.71 ± 0.23^{c}	0.92	2.92 ± 0.23^{b}	0.98
Residue	1.33 ± 0.09^{a}	0.95	1.63 ± 0.19^{a}	0.82
n-butanol-partitioned	1.85 ± 0.06^{b}	0.99	1.92 ± 0.06^{a}	0.86

*r^2= regression coefficient

Values are mean of three replicates ± SEM; Values with different superscripts are significantly different from others ($p < 0.05$)

4.3 Fasting Blood Glucose of Diabetic Rats Orally Administered 25 mg/kg Body Weight of Aqueous and Solvent-partitioned Fractions of Aqueous Extract of *Chrysophyllum albidum* Stem Bark

Compared with distilled water treated non-diabetic rats, administration of alloxan significantly ($p < 0.05$) increased the FBG level of the rats (Figure 18). Administration of 25 mg/kg body weight of the aqueous and ethyl acetate-partitioned extracts were not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats while the residue and n-butanol treated groups were not significantly ($p < 0.05$) different from the distilled water treated diabetic rats.

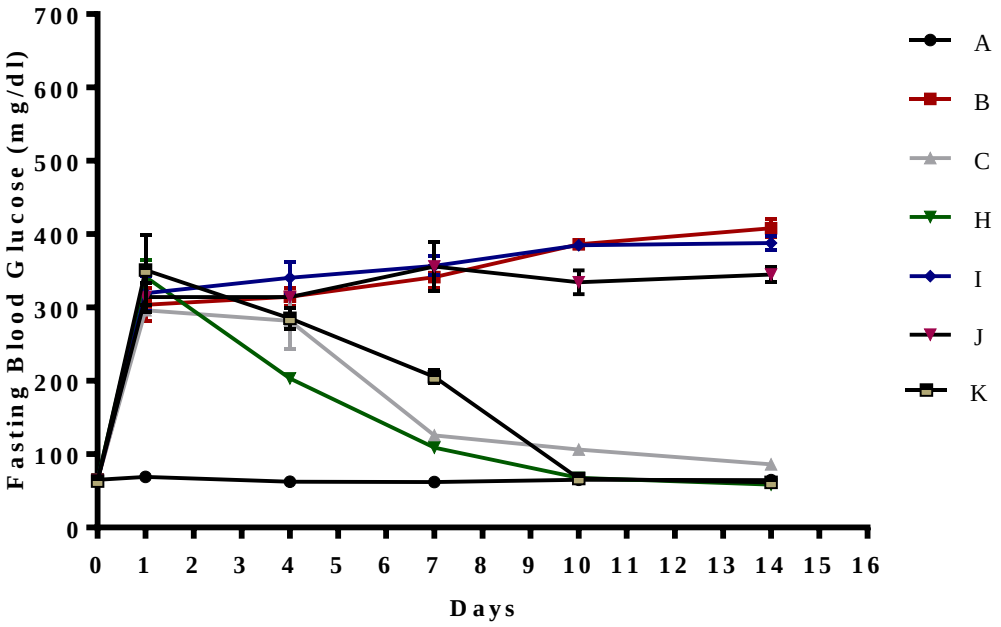


Figure 16: Fasting Blood Glucose of Diabetic Rats Orally Administered 25 Mg/Kg Body Weight of Aqueous and Solvent-partitioned Extracts of *Chrysophyllum albidum* Stem Bark

Where:

A = Non diabetic rats + 1mL distilled water

B = Diabetic rats + 1mL distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

H = Diabetic rats + 25 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

I = Diabetic rats + 25 mg/kg body weight of the residue of aqueous extract of *C. albidum* stem bark

J = Diabetic rats + 25 mg/kg body weight of n-buthanol-partitioned fraction of aqueous extract of *C. albidum* stem bark

K = Diabetic rats + 25 mg/kg body weight of aqueous extract of *C. albidum* stem bark.

4.4 *In vivo* Anti-diabetic Study of Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark in Alloxan-Induced Diabetic Rats

4.4.1 Fasting Blood Glucose Levels of Diabetic Rats Orally Administered Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark

Compared with distilled water treated diabetic rats, Administration of 2.5, 5, 10 and 20 mg/kg body weight of ethyl acetate-partitioned fraction of *Chrysophyllum albidum* stem bark significantly ($p< 0.05$) decreased the FBG level of the diabetic rats (Figure 19). The 2.5 mg/kg body weight of ethyl acetate-partitioned fraction of *Chrysophyllum albidum* stem bark was not significantly ($p< 0.05$) different from the distilled water treated non-diabetic rats while 5, 10 and 20 mg/kg body weight of the fraction were not significantly ($p< 0.05$) different from the glibenclamide treated diabetic rats.

4.4.2 Effect of Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark on Hepatic Glucose and Glycogen in Alloxan-induced Diabetic Rats

Administration of alloxan significantly ($p < 0.05$) increased the hepatic glucose and decreased glycogen content when compared with the distilled water treated non-diabetic rats (Figure 20). Oral administration of 2.5, 5, 10 and 20 mg/kg body weight of the ethyl acetate-partitioned fraction of *Chrysophyllum albidum* stem bark however significantly ($p < 0.05$) decreased the hepatic glucose and increased the glycogen content.

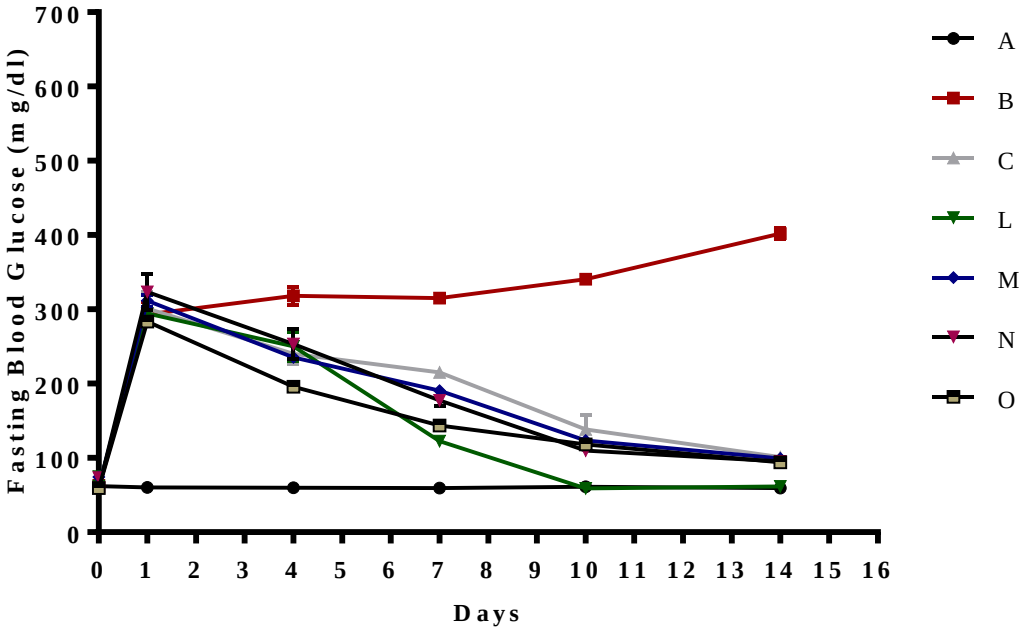


Figure 17: Fasting Blood Glucose Levels of Diabetic Rats Orally Administered Doses of Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

L = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

M = Diabetic rats + 5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

N = Diabetic rats + 10 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

O = Diabetic rats + 20 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

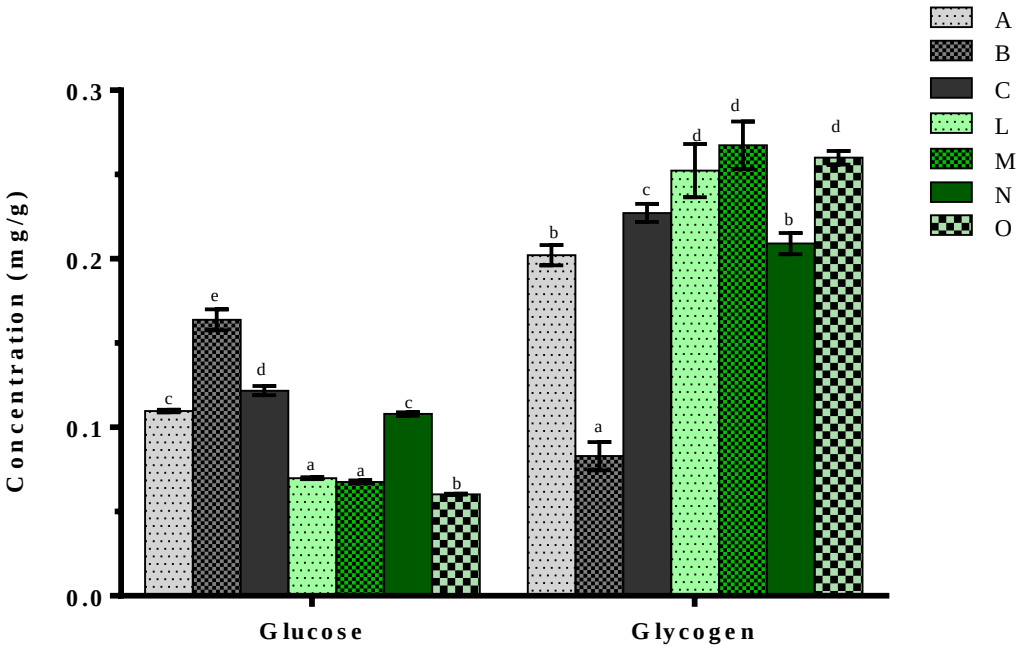


Figure 18: Hepatic Glucose and Glycogen Level of Diabetic Rats Orally Administered Ethyl Acetate-partition Fraction of *Chrysophyllum albidum* Stem Bark

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

L = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

M = Diabetic rats + 5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

N = Diabetic rats + 10 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

O = Diabetic rats + 20 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

4.4.3 Effect of Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark on the Activity of Alpha Amylase in Alloxan-induced Diabetic Rats

Administration of alloxan significantly ($p < 0.05$) increased the α-amylase activity of the liver, small intestine and pancreas when compared with distilled water treated non-diabetic rats (Figure 21). Oral administration of 2.5, 5, 10 and 20 mg/kg body weight of ethyl acetate-partitioned fraction of *Chrysophyllum albidum* stem bark significantly ($p < 0.05$) decreased the alloxan-induced increase in activity of alpha amylase in all tissues. Only 2.5 mg/kg body weight of the fraction was not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats in the liver and small intestine while 5 and 10 mg/kg body weight treatments were not significantly ($p < 0.05$) different from the glibenclamide treated diabetic rats in the small intestine.

4.4.4 Effect of Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark on the Activity of Glucose-6-phospate Dehydrogenase in Alloxan-induced Diabetic Rats

Alloxan administration significantly ($p < 0.05$) decreased glucose-6-phospate dehydrogenase activity in the liver when compared with distilled water treated non-diabetic rats (Figure 22). Oral administration of 2.5, 5, 10 and 20 mg/kg body weight of ethyl acetate-partitioned fraction of *Chrysophyllum albidum* stem bark significantly ($p < 0.05$) increased the activity of glucose-6-phospate dehydrogenase and were not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats except 5 mg/kg body weight treatment which did not compared well with either distilled water treated non-diabetic rats or the reference drug treated diabetic rats.

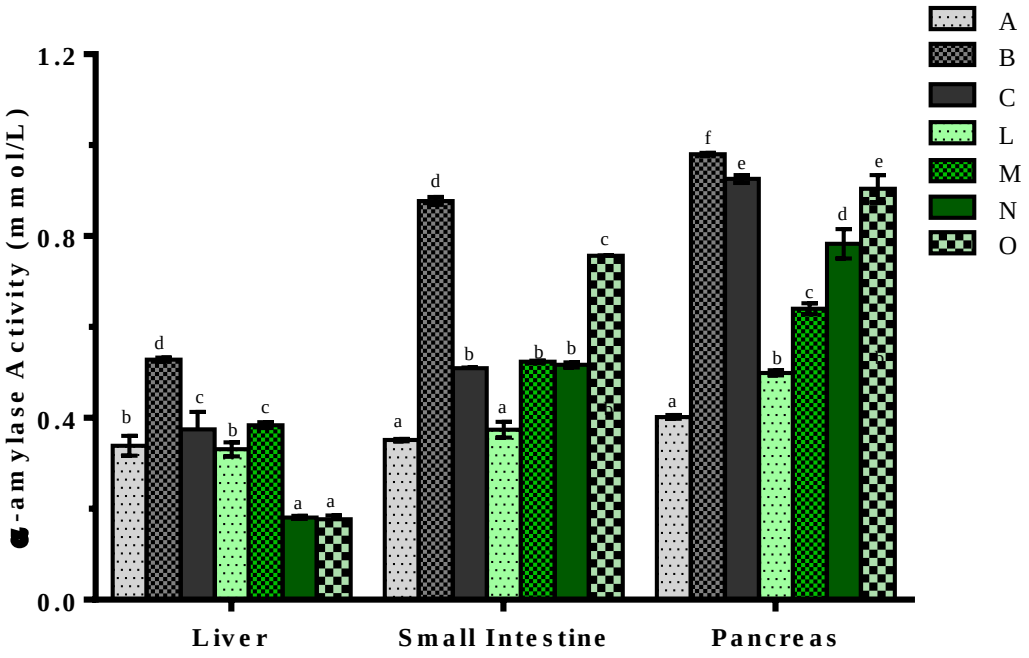


Figure 19: Activity of Alpha Amylase in the Liver , Small Intestine and Pancreas of Diabetic Rats Orally Administered Ethyl Acetate-partition Fraction of *Chrysophyllum albidum* Stem Bark

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

L = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

M = Diabetic rats + 5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

N = Diabetic rats + 10 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

O = Diabetic rats + 20 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

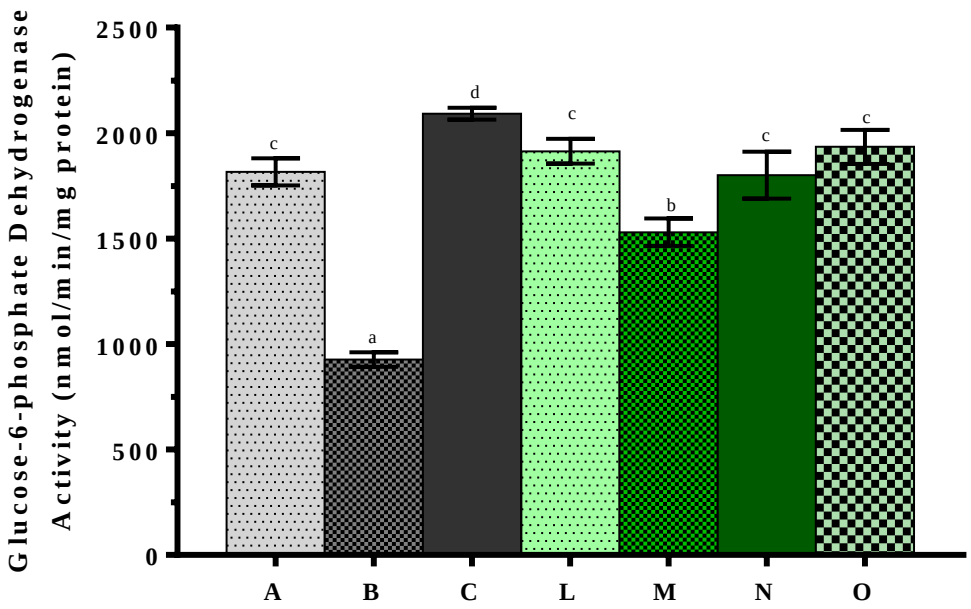


Figure 20: Activity Of Glucose-6-Phosphate Dehydrogenase In the Liver of Diabetic Rats Orally Administered Ethyl Acetate-partition Fraction of *Chrysophyllum albidum* Stem Bark

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

L = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

M = Diabetic rats + 5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

N = Diabetic rats + 10 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

O = Diabetic rats + 20 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

4.4.5 Effect of Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark on the Serum Lipids in Alloxan-induced Diabetic Rats

Compared with distilled water treated non-diabetic rats, alloxan administration significantly ($p < 0.05$) increased the serum cholesterol and triglycerides and decreased the high density lipoprotein (HDL) level. Administration of doses of ethyl acetate-partition fraction decreased the serum cholesterol and triglycerides and increased the serum HDL level though, only the 2.5 mg/kg body weight treatment was not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats (Figure 23).

4.4.6 Effect of Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark on the Malondialdehyde Level in Alloxan-induced Diabetic Rats

Alloxan administration significantly increased ($p < 0.05$) MDA level (Figure 24) when compared with distilled water treated non-diabetic rats. Treatment with 2.5, 5, 10 and 20 mg/kg body weight of ethyl acetate-partitioned fraction significantly ($p < 0.05$) reduced the serum MDA level but only 2.5 mg/kg body weight treatment was not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats.

4.4.7 Effect of Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark on Antioxidant Enzymes Activity in Alloxan-induced Diabetic Rats

Compared with distilled water treated non-diabetic rats, administration of alloxan significantly ($p < 0.05$) reduced superoxide dismutase (SOD) (Figure 25) and catalase (Figure 26) activities in the liver. Treatment with 2.5 mg/kg body weight of ethyl acetate-partitioned fraction significantly ($p < 0.05$) increased the activity of the enzymes and was not different from the distilled water treated non-diabetic rats. Catalase activity of the 5, 10 and 20 mg/kg body weight treatment were not different from glibenclamide treated diabetic rats while SOD activity of the glibenclamide and 20 mg/kg body weight treatments were not different from the distilled water treated diabetic rats.

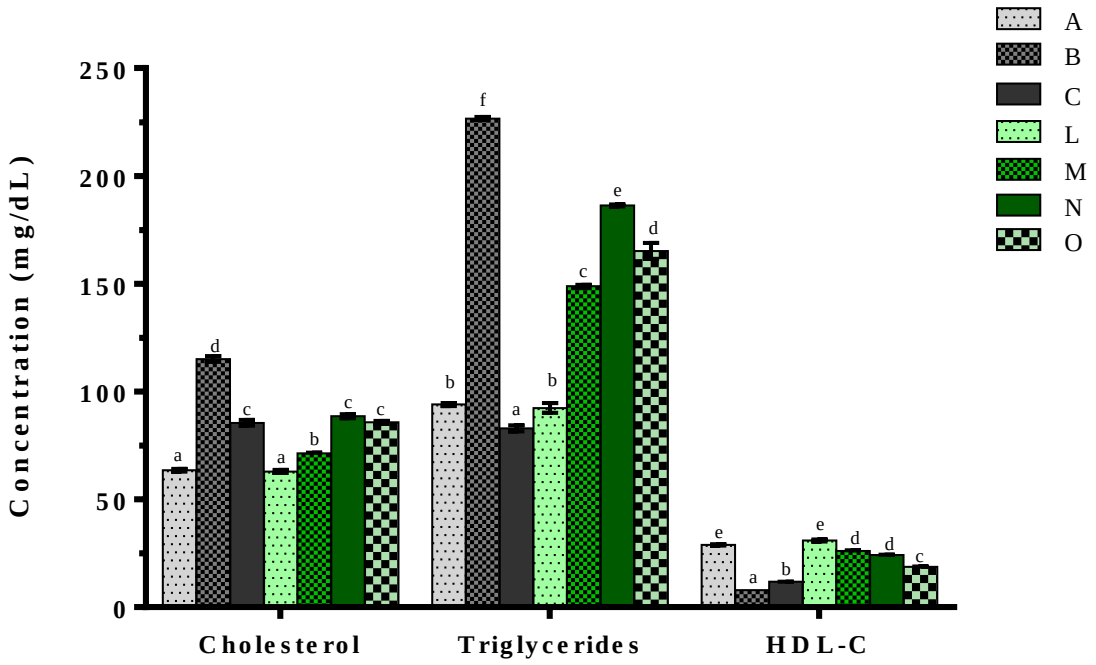


Figure 21: Serum Lipid Profile of Diabetic Rats Orally Administered Ethyl Acetate-Partition Fraction of *Chrysophyllum albidum* Stem Bark

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

L = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

M = Diabetic rats + 5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

N = Diabetic rats + 10 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

O = Diabetic rats + 20 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

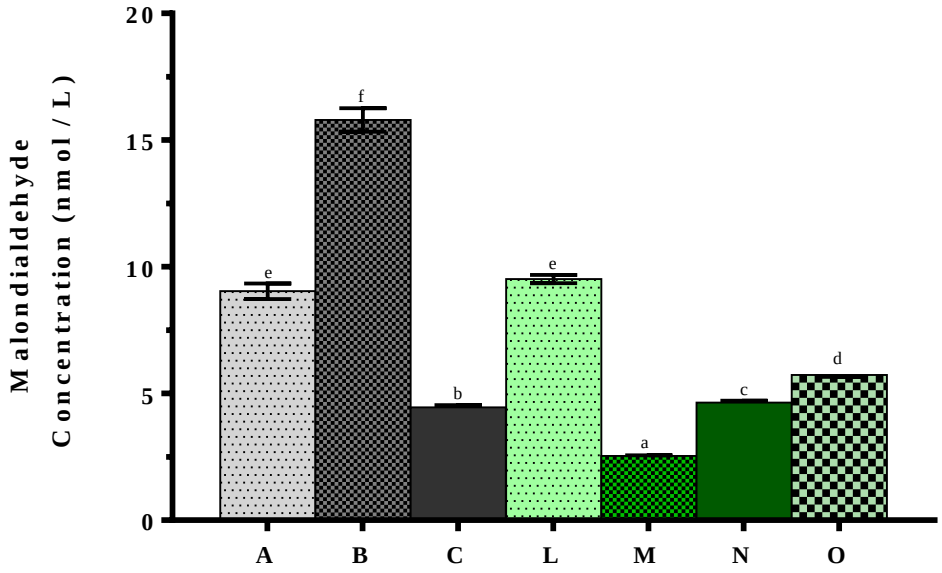


Figure 22: Malondialdehyde Levels of Diabetic Rats Orally Administered Ethyl Acetate-partitioned Fraction from *Chrysophyllum albidum* Stem Bark

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

L = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

M = Diabetic rats + 5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

N = Diabetic rats + 10 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

O = Diabetic rats + 20 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

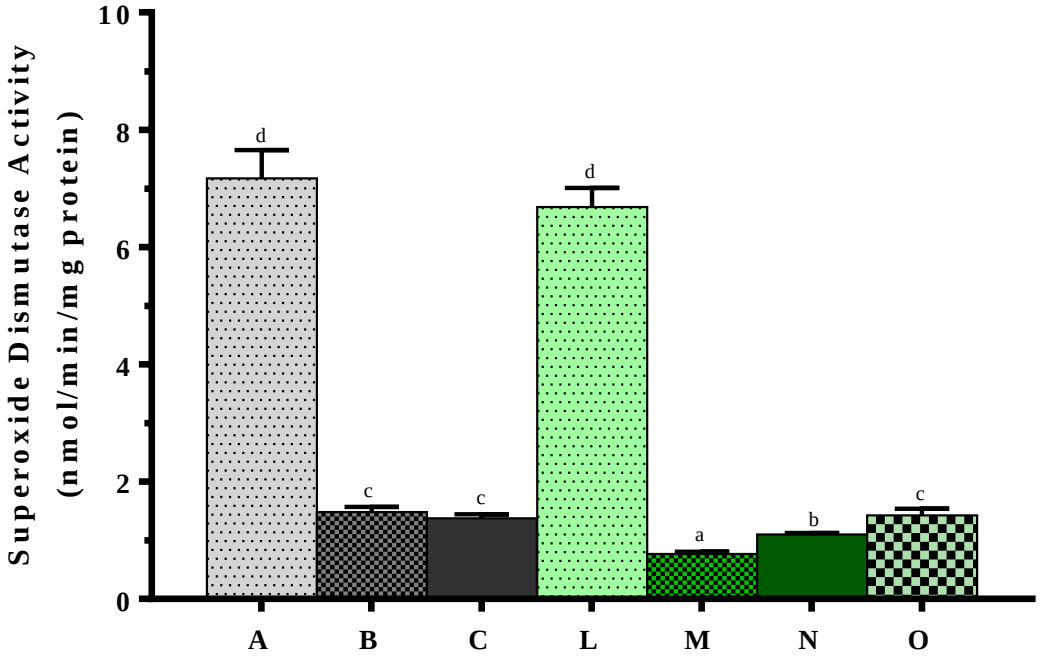


Figure 23: Activity of Superoxide Dismutase in the Liver of Diabetic Rats Orally Administered Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

L = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

M = Diabetic rats + 5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

N = Diabetic rats + 10 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

O = Diabetic rats + 20 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

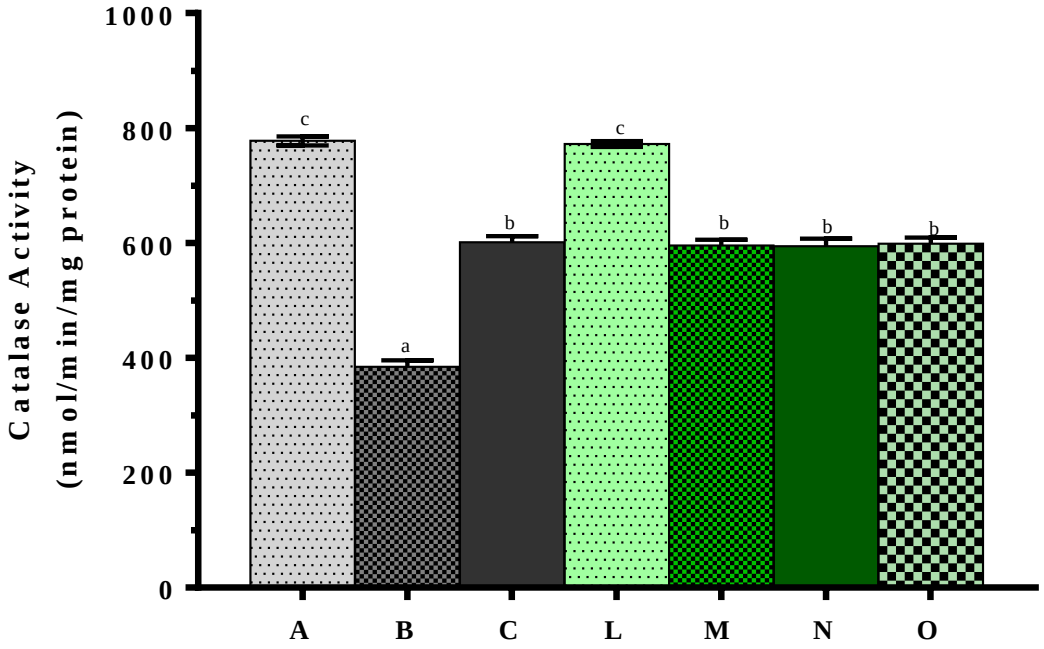


Figure 24: Activity of Catalase in Liver of Diabetic Rats Orally Administered Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

L = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

M = Diabetic rats + 5 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

N = Diabetic rats + 10 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

O = Diabetic rats + 20 mg/kg body weight of ethyl acetate-partitioned fraction of aqueous extract of *C. albidum* stem bark

4.5 Column and Thin Layer Chromatography of Ethyl Acetate-partitioned Fraction obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark

Column chromatographic analysis of ethyl acetate-partitioned fraction produced eleven fractions coded F1-F11 (Table 8). The fractions were pooled together into seven as F1, F3, F4, F5, F7, F9 and F10 based on R_f values using TLC analysis (plate 2).

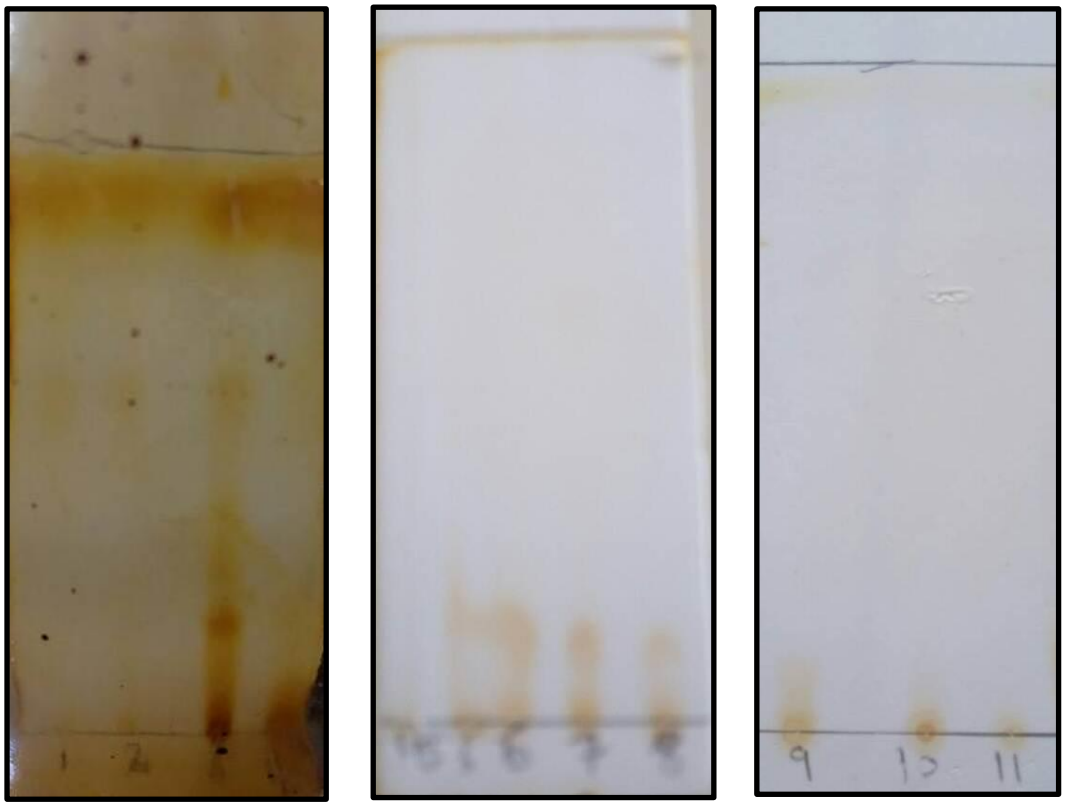


Plate 2: Chromatogram of Ethyl Acetate-Partitioned Fractions Visualized with Iodine Vapour

Table 8: Pooled Fractions from TLC Chromatogram of Fractions of Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark

Fractions	Spots	R_f Values	Adopted nomenclature
F_{1-2}	1	0.6	F_1
F_3	3	0.2, 0.6, 0.8	F_3
F_4	1	0.8	F_4
F_{5-6}	1	0.2	F_5
F_{7-8}	1	0.2	F_7
F_9	1	0.1	F_9
F_{10-11}	0	0	F_{10}

4.6 *In vivo* Anti-diabetic Activity of Column Chromatographic Fractions of Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem bark in Alloxan-induced Diabetic Rats

4.6.1 Fasting Blood Glucose Levels of Diabetic Rats Orally Administered Chromatographic Fractions obtained from Ethyl Acetate-partitioned Fraction from *Chrysophyllum albidum* Stem Bark

Compared with distilled water treated non-diabetic rats, alloxan administration significantly ($p < 0.05$) increased the FBG level of the rats (Figure 27). Among the seven column chromatographic fractions administered (F1, F3, F4, F5, F7, F9 and F10) to the diabetic groups at 2.5 mg/kg body weight, only F5 and F7 significantly ($p < 0.05$) reduced the FBG levels of their respective groups. By day 10, the F5 treated group was not significantly ($p < 0.05$) from the glibenclamide treated diabetic rats while F7 was not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats. Other fractions were not significantly ($p < 0.05$) different from the distilled water treated diabetic rats.

4.6.2 Effect of Chromatographic Fractions from Ethyl Acetate-partitioned Fraction obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark on Body Weight of Alloxan-Induced Diabetic Rats

Compared with distilled water treated non-diabetic rats, alloxan administration significantly ($p < 0.05$) decreased the body weight of the rats which continued progressively till the end of the experiment (Figure 28). The F5, F7 and glibenclamide treatments significantly ($p < 0.05$) increased the body weight of the rats and were not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats.

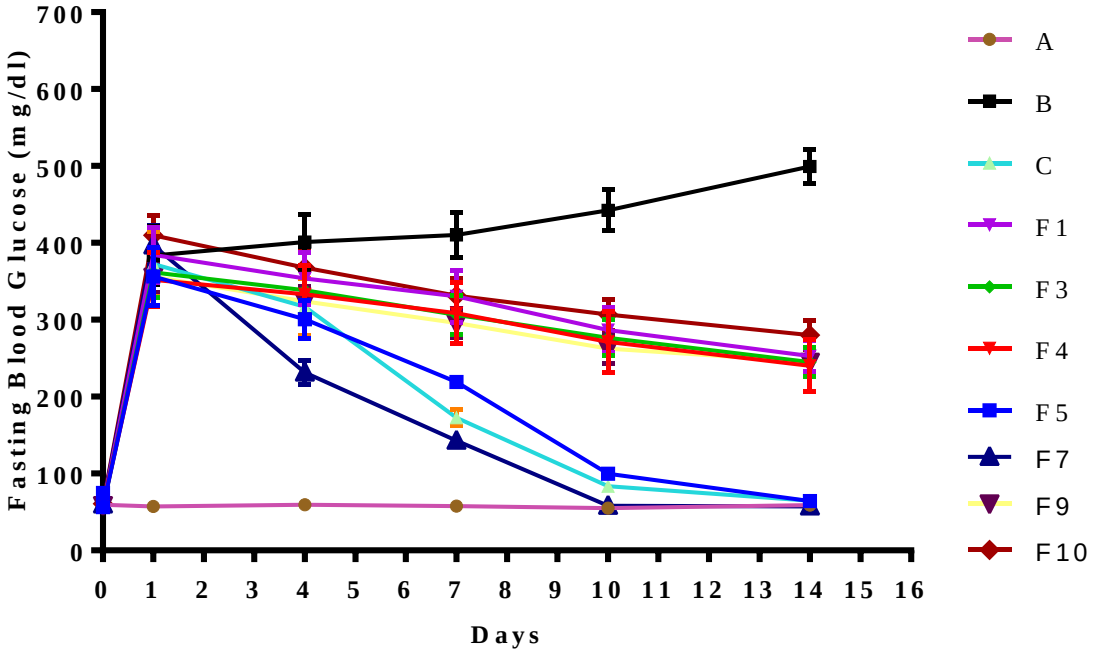


Figure 25: Fasting Blood Glucose Levels of Diabetic Rats Orally Administered Chromatographic Fractions obtained from Ethyl Acetate-partitioned Fraction from *Chrysophyllum albidum* Stem Bark

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

F1 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 1

F3 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 3

F4 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 4

F5 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 5

F7 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 7

F9 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 9

F10 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 10

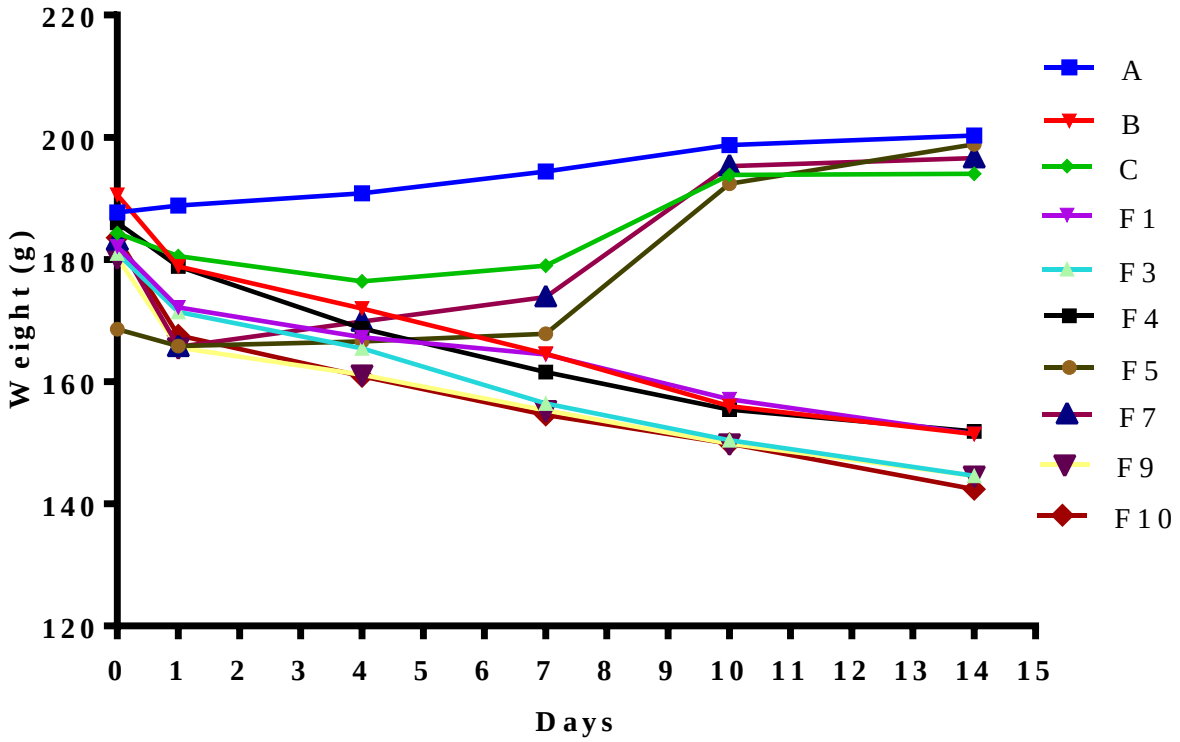


Figure 26: Body Weight of Alloxan-induced Diabetic Rats Orally Administered Chromatographic Fractions obtained from Ethyl Acetate-partitioned Fraction from *Chrysophyllum albidum* Stem Bark

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

F1 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 1

F3 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 3

F4 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 4

F5 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 5

F7 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 7

F9 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 9

F10 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 10

4.6.3 Effect of Chromatographic Fractions from Ethyl Acetate-partitioned Fraction obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark on Feed and Water Intake of Alloxan-induced Diabetic Rats

Alloxan administration significantly ($p < 0.05$) increased the feed and water intake of experimental rats when compared with distilled water treated non-diabetic rats (Table 9). The F5 and F7 among the treatments significantly ($p < 0.05$) decreased the feed and water intake though, only F7 was not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats by the week 2 of the experiment.

4.6.4 Effect of Chromatographic Fractions from Ethyl Acetate-partitioned Fraction obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark on Oral Glucose Tolerance Test of Alloxan-induced Diabetic Rats

At 0 minute the glucose level of the rats ranged from 55.86 to 197.71 while that of the distilled water treated diabetic rats was 354.86. Thirty minutes later, glucose level of all the groups significantly ($p < 0.05$) increased when compared with the distilled water treated non-diabetic rats and by 120 minutes, only the blood glucose level of the F7 treated diabetic rats returned to the basal level and was not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats (Table 10).

Table 9: Feed and Water Intake of Alloxan-induced Diabetic Rats Orally Administered Chromatographic Fractions obtained from Ethyl Acetate-partitioned Fraction of Aqueous Extract of *Chrysophyllum albidum* Stem Bark

Treatment groups	Feed Intake (g)		Water Intake (mL)	
	Week 1	Week 2	Week 1	Week 2
Non diabetic rats + 1mL distilled water	290.12 ± 4.06^{a}	296.80 ± 2.26^{b}	148.03 ± 0.49^{a}	155.18 ± 2.92^{a}
Diabetic rats + 1mL distilled water	326.68 ± 6.33^{c}	330.61 ± 3.12^{d}	189.30 ± 5.51^{c}	210.86 ± 5.75^{c}
Diabetic rats + 2.5 mg/kg body weight of glibenclamide	326.51 ± 1.82^{c}	262.94 ± 3.55^{a}	183.82 ± 2.01^{c}	162.94 ± 3.55^{a}
Diabetic rats + 2.5 mg/kg body weight of EACF 1	320.10 ± 5.19^{c}	327.61 ± 2.81^{d}	194.25 ± 1.71^{d}	207.61 ± 2.81^{c}
Diabetic rats + 2.5 mg/kg body weight of EACF 3	314.08 ± 4.44^{c}	317.59 ± 6.60^{c}	193.33 ± 2.14^{d}	207.59 ± 6.60^{c}
Diabetic rats + 2.5 mg/kg body weight of EACF 4	$318.96 \pm 8.56^{b, c}$	337.71 ± 4.02^{d}	194.55 ± 4.07^{d}	214.26 ± 1.57^{c}
Diabetic rats + 2.5 mg/kg body weight of EACF 5	313.66 ± 8.90^{b}	294.41 ± 3.82^{b}	185.73 ± 2.81^{c}	162.02 ± 1.88^{b}
Diabetic rats + 2.5 mg/kg body weight of EACF 7	304.53 ± 4.48^{b}	289.86 ± 2.15^{b}	164.92 ± 6.58^{b}	157.70 ± 2.42^{a}
Diabetic rats + 2.5 mg/kg body weight of EACF 9	318.58 ± 2.06^{c}	331.61 ± 3.36^{d}	191.70 ± 1.66^{d}	212.25 ± 3.29^{c}
Diabetic rats + 2.5 mg/kg body weight of EACF 10	328.92 ± 2.44^{c}	347.54 ± 4.09^{e}	192.12 ± 2.56^{d}	211.37 ± 1.32^{c}

Values are mean of three replicates ± SEM.

Values with different superscripts are significantly ($p < 0.05$) different from others.

Where EACF = ethyl acetate chromatographic fraction

Table 10: Oral Glucose Tolerance of Alloxan-induced Diabetic Rats Orally Administered Some Chromatographic Fractions obtained From Ethyl Acetate-partitioned Fraction from *Chrysophyllum Albidum* Stem Bark

	Time (Minutes)				
Treatment Group	0	30	60	90	120
Non diabetic rats + 1mL of distilled water	55.86 ± 4.53^{a}	81 ± 5.48^{a}	62.71 ± 2.69^{a}	58.71 ± 1.60^{a}	55.86 ± 4.02^{a}
Diabetic rats + 1mL of distilled water	354.86 ± 33.58^{c}	522 ± 34.76^{e}	483.57 ± 41.19^{e}	466.86 ± 41.06^{a}	419.71 ± 33.46^{d}
Diabetic rats + 2.5 mg/kg body weight of glibenclamide	157.57 ± 28.50^{b}	340.14 ± 46.98^{c}	295.86 ± 41.5^{c}	252.57 ± 28.60^{c}	224.57 ± 24.41^{b}
Diabetic rats + 2.5 mg/kg body weight of EACF 5	197.71 ± 19.35^{b}	428.29 ± 52.88^{d}	371.29 ± 52.72^{d}	342.14 ± 49.38^{d}	288.71 ± 27.44^{c}
Diabetic rats + 2.5 mg/kg body weight of EACF 7	55.86 ± 3.98^{a}	254.57 ± 14.55^{b}	199.14 ± 12.13^{b}	105.14 ± 13.17^{b}	61.43 ± 10.85^{a}

Values are mean of ten replicates ± SEM.

Values with different superscripts are significantly different from others ($p < 0.05$).

Where EACF = ethyl acetate chromatographic fraction

4.6.5 Glucose and Glycogen Concentrations of Alloxan-Induced Diabetic Rats Orally Administered Chromatographic Fractions obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark

Compared with distilled water treated non-diabetic rats, alloxan administration significantly ($p < 0.05$) increased the hepatic glucose and decreased the glycogen level (Figure 29). Although oral administration of 2.5 mg/kg body weight of F5 and F7 significantly ($p < 0.05$) reduced the hepatic glucose and increased the glycogen level of the diabetic rats, only F7 was not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats. The glycogen content of the F5 treated group was not significantly ($p < 0.05$) different from the glibenclamide treated diabetic rats.

4.6.6 Effect of Chromatographic Fractions from Ethyl Acetate-partitioned Fraction obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark on Serum Insulin of Alloxan-induced Diabetic Rats

Alloxan administration significantly ($p < 0.05$) decreased the serum insulin level when compared with distilled water treated non-diabetic rats (Figure 30). Administrations of ethyl acetate chromatographic fractions significantly ($p < 0.05$) increased serum insulin of the F7 treated diabetic rats only. The F5 treated group was not significantly ($p < 0.05$) different from distilled water treated diabetic rats.

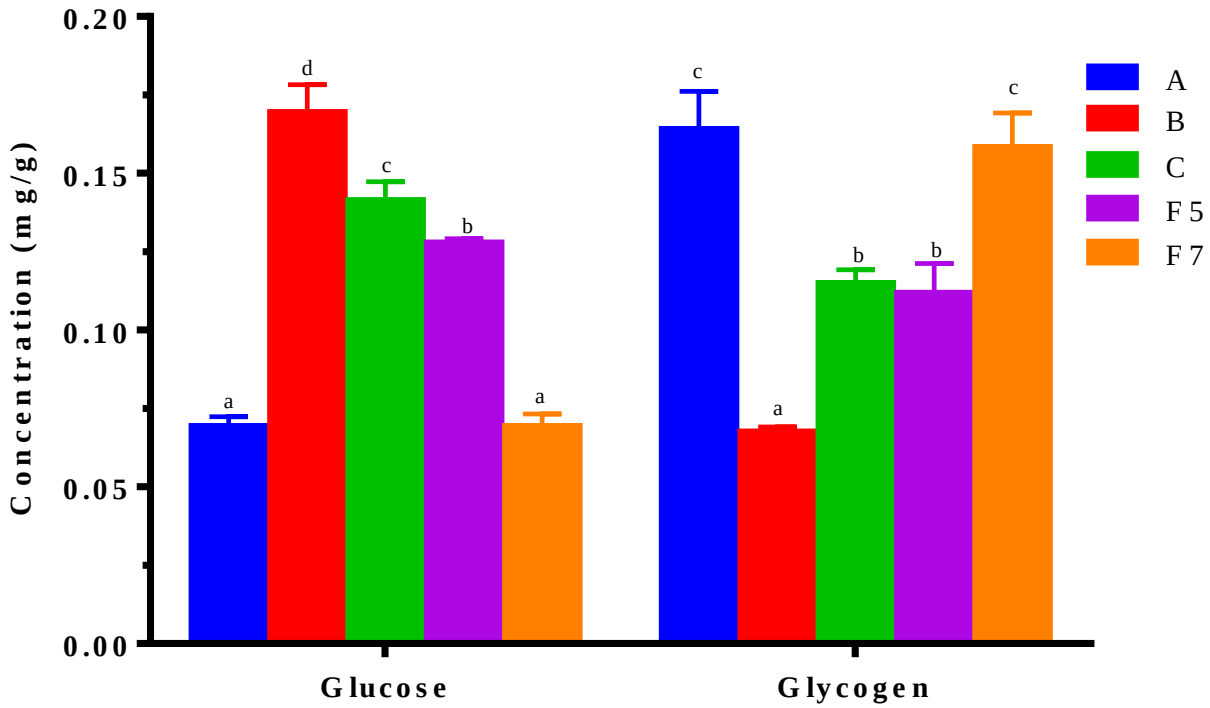


Figure 27: Glucose and Glycogen Concentrations of Alloxan-induced Diabetic Rats Orally Administered Chromatographic Fractions obtained from Ethyl Acetate-partitioned Fraction from *Chrysophyllum albidum* Stem Bark.

Bars with different superscripts are significantly ($p < 0.05$) different from others.

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

F5 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 5

F7 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 7

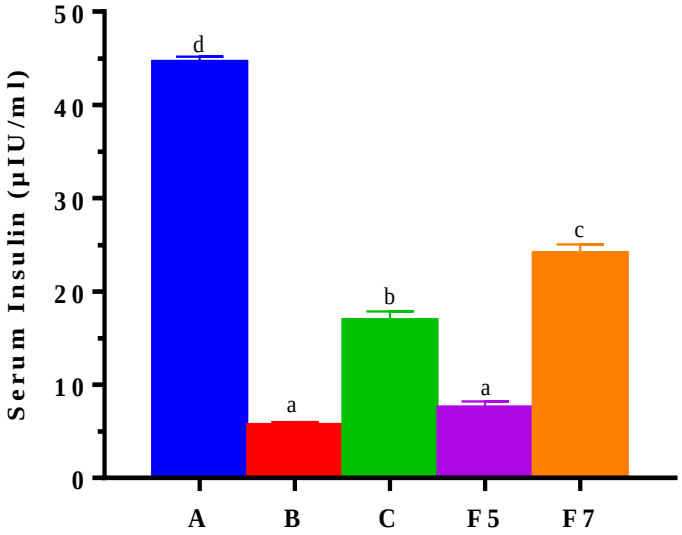


Figure 28: Serum insulin level of alloxan-induced Diabetic Rats Orally Administered Chromatographic Fractions obtained from Ethyl Acetate-partitioned Fraction from *Chrysophyllum albidum* Stem Bark

Bars with different superscripts are significantly ($p < 0.05$) different from others

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

F5 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 5

F7 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 7

4.6.7 Effect of Chromatographic Fractions from Ethyl Acetate-partitioned Fraction obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark on Hepatic Glucokinase Activity of Alloxan-induced Diabetic Rats

Alloxan administration significantly ($p < 0.05$) decreased the activity of glucokinase when compared with distilled water treated non-diabetic rats (Figure 31). However, administration of chromatographic fractions of ethyl acetate-partitioned fraction significantly ($p < 0.05$) increased the enzyme activity. The F5 treated diabetic rats were not significantly ($p < 0.05$) different from the glibenclamide treated diabetic rats while F7 treated diabetic rats were not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats.

4.6.8 Effect of Chromatographic Fractions of Ethyl Acetate-partitioned Fraction obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark on the Activity of Glucose-6-phosphatase and Fructose-1, 6-bisphosphatase Enzymes in Alloxan-induced Diabetic Rats

Alloxan administration significantly ($p < 0.05$) increased the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase when compared with distilled water treated non-diabetic rats (Figure 32). Treatment with chromatographic fractions of ethyl acetate-partitioned fractions significantly ($p < 0.05$) decreased the activity of the enzymes. The F5 treated diabetic group was not significantly ($p < 0.05$) different from the glibenclamide treated diabetic group while F7 treated diabetic rats were not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats activity of glucose-6-phosphatase in both fractions compared better than the reference drug treated diabetic rats, they did not compared well with distilled water treated non-diabetic rats.

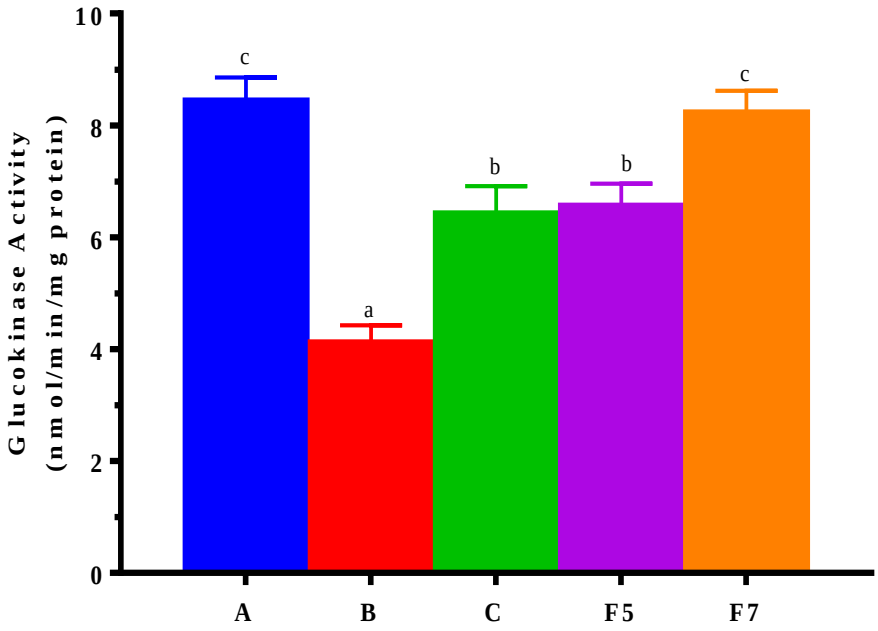


Figure 29: Glucokinase activity of Alloxan-induced Diabetic Rats Orally Administered Chromatographic Fractions obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark

Bars with different superscripts are significantly different from each other ($p < 0.05$).

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

F5 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 5

F7 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 7

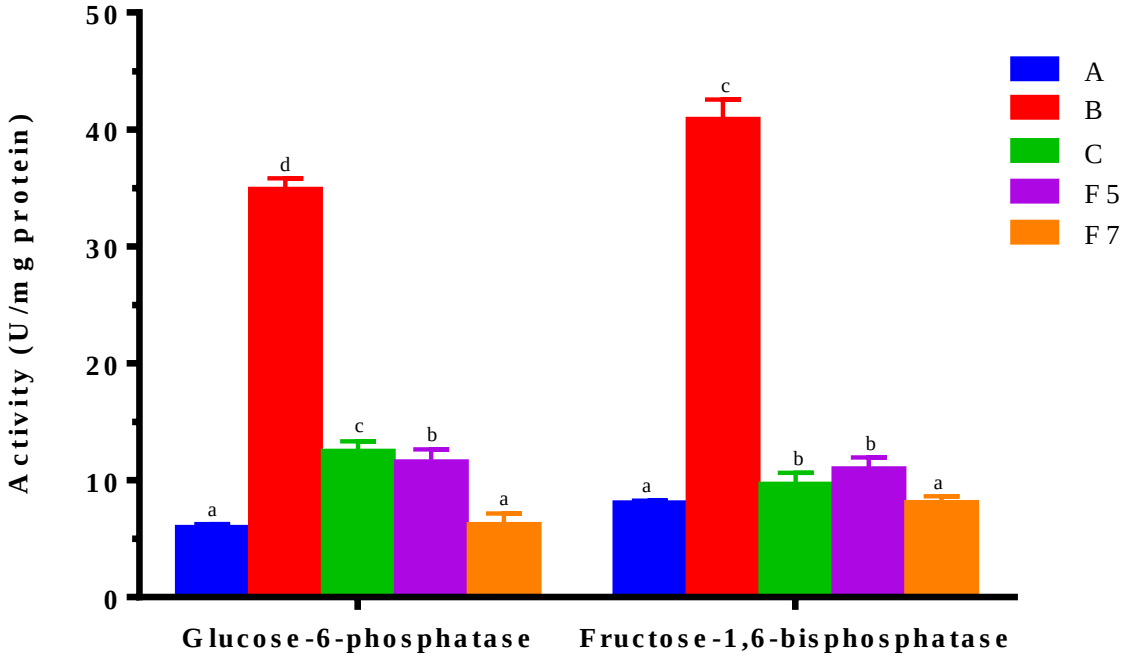


Figure 30: Activity of Glucose-6-phosphatase and Fructose 1,6-bisphosphatase of Alloxan-induced Diabetic Rats Orally Administered Chromatographic Fractions obtained from Ethyl Acetate-partitioned Fraction from *Chrysophyllum albidum* Stem Bark

Bars with different superscripts are significantly different from each other ($p < 0.05$).

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

F5 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 5

F7 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 7

4.6.9 Effect of Chromatographic Fractions from Ethyl Acetate-partitioned Fraction obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark on Hepatic Glucose-6-phosphate Dehydrogenase Activity in Alloxan-induced Diabetic Rats

Alloxan administration significantly ($p < 0.05$) decreased the activity of glucose-6-phosphate dehydrogenase when compared with distilled water treated non-diabetic rats (Figure 33). Administration of chromatographic fractions of ethyl acetate-partitioned fractions significantly ($p < 0.05$) increased the activity of the enzyme and both F5 and F7 were not significantly ($p < 0.05$) different from the distilled water treated diabetic rats.

4.6.10 Effect of Chromatographic Fractions from Ethyl Acetate-partitioned Fraction obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark on C-reactive Protein in Alloxan-induced Diabetic Rats

Alloxan administration significantly ($p < 0.05$) increased the serum C-reactive protein level of the rats when compared with the distilled water treated non-diabetic rats (Figure 34). Administration of chromatographic fractions of ethyl acetate-partitioned fraction significantly ($p < 0.05$) decreased serum CRP level though, only F7 treated diabetic rats was not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats.

4.6.11 Effect of Chromatographic Fractions of Ethyl Acetate-partitioned Fractions obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark on Glycosylated Haemoglobin of Alloxan-induced Diabetic Rats

Compared with the distilled water treated non-diabetic rats, alloxan administration significantly ($p < 0.05$) increased the serum glycosylated haemoglobin of the rats (Figure 35). Oral administration of F5 and F7 significantly ($p < 0.05$) reduced the alloxan-induced increase in serum glycosylated haemoglobin. Although, both fractions reduced the serum glycosylated haemoglobin of the rats, only F7 treated rats was not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats.

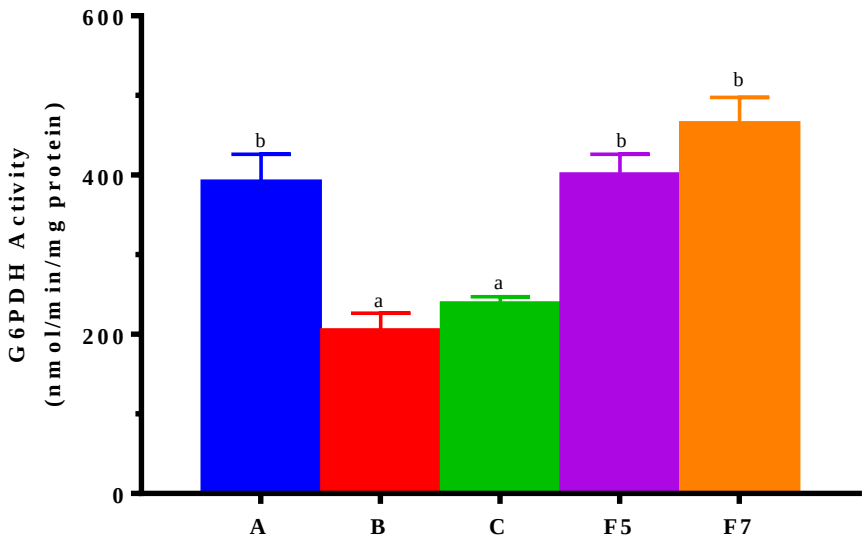


Figure 31: Hepatic G6PDH level of Alloxan-induced Diabetic Rats Orally Administered Chromatographic Fractions obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark

Bars with different superscripts are significantly different from each other ($p < 0.05$).

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

F5 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 5

F7 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 7

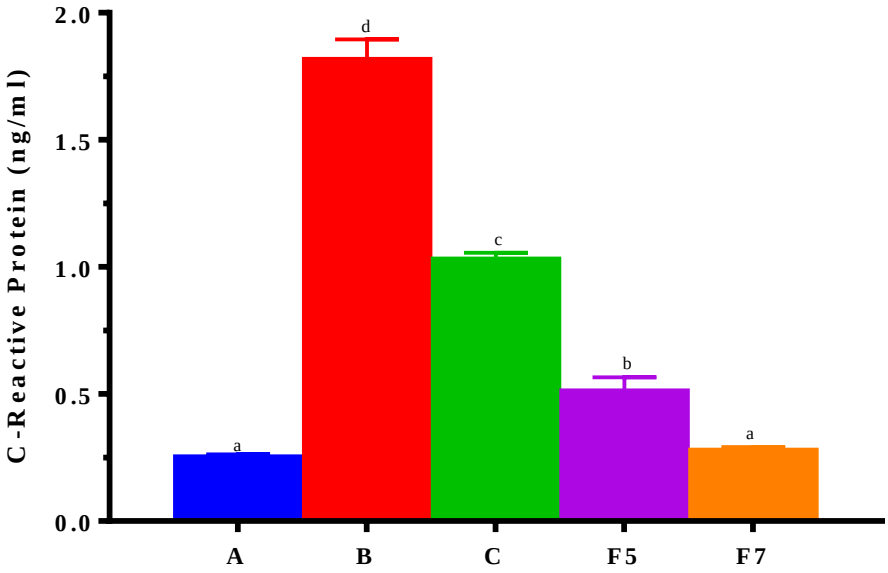


Figure 32: C-reactive Protein of Alloxan-induced Diabetic Rats Orally Administered Chromatographic Fractions obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

F5 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 5

F7 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 7

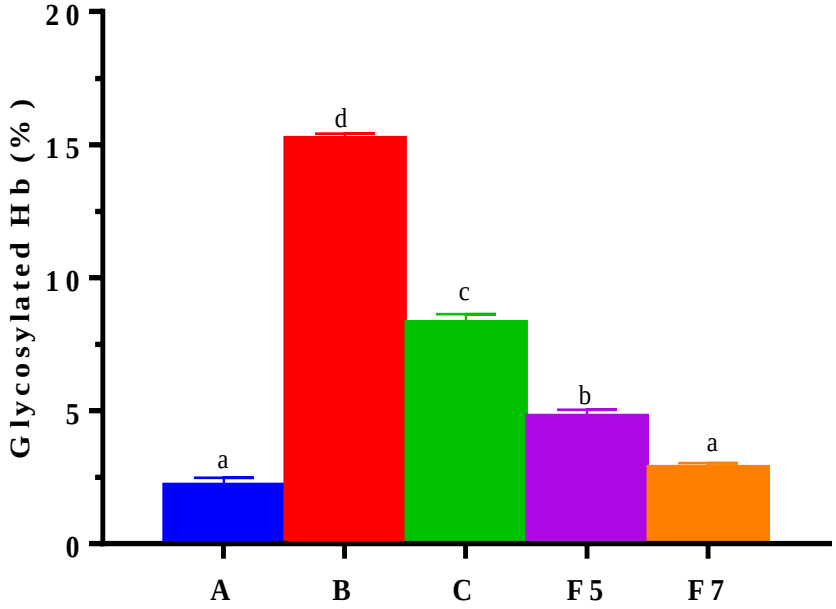


Figure 33: Glycosylated Haemoglobin of Alloxan-induced Diabetic Rats Orally Administered Chromatographic Fractions obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark

Bars with different superscripts are significantly different from each other ($p < 0.05$).

Where:

A = Non diabetic rats + 1mL of distilled water

B = Diabetic rats + 1mL of distilled water

C = Diabetic rats + 2.5 mg/kg body weight of glibenclamide

F5 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 5

F7 = Diabetic rats + 2.5 mg/kg body weight of ethyl acetate chromatographic fraction 7

4.6.12 Effect of Chromatographic Fractions from Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark on Serum Lipids of Alloxan-induced Diabetic Rats

Compared with the distilled water treated non-diabetic rats, alloxan administration significantly ($p < 0.05$) increased the serum cholesterol, triglycerides (TG), low density lipoprotein-cholesterol (LDL-C), very low density lipoprotein-cholesterol (VLDL-C), atherogenic index (AI) and significantly ($p < 0.05$) decreased the high density lipoprotein-cholesterol (HDL-C) (Table 11). Oral administration of both F5 and F7 significantly ($p < 0.05$) increased HDL-C and decreased other parameters. The serum triglycerides, low density lipoprotein-cholesterol, very low density lipoprotein-cholesterol, atherogenic index and high density lipoprotein-cholesterol of both fractions were not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats.

4.6.13 Effect of Chromatographic Fractions of Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark on Antioxidant Enzymes and Malondialdehyde Levels in Alloxan-induced Diabetic Rats

Alloxan administration significantly ($p < 0.05$) increased the malondialdehyde (MDA) level and decreased the catalase and superoxide dismutase (SOD) activities **(**Table 12). Oral administration of chromatographic fractions of ethyl acetate-partitioned from C*hrysophyllum albidum* stem bark significantly ($p < 0.05$) reduced the MDA level and increased the SOD and catalase activities of the treated rats.

The MDA level of F5, F7 and the glibenclamide treated diabetic rats were not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats. Catalase and SOD activities of the F7 treated diabetic rats were not significantly ($p < 0.05$) different from the distilled water treated non-diabetic rats while SOD activity of F5 treated diabetic rats was not significantly ($p < 0.05$) different from the glibenclamide treated diabetic rats.

Table 11: Serum Lipids of Alloxan-induced Diabetic Rats Orally Administered Chromatographic Fractions obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark

Treatment Group	Cholesterol	Triglyceride	HDL-C	LDL-C	VLDL-C	Atherogenic index (LDL-C/ HDL-C)
Non-diabetic rats + 1mL of distilled water	26.12 ± 0.5^{d}	4.14 ± 0.5^{a}	16.69 ± 1.5^{d}	8.60 ± 1.6^{c}	0.83 ± 0.1^{a}	0.52 ± 0.1^{b}
Diabetic rats + 1mL of distilled water	57.92 ± 1.0^{e}	26.98 ± 0.9^{c}	3.55 ± 0.6^{a}	48.98 ± 1.2^{d}	5.40 ± 0.2^{c}	14.12 ± 2.5^{c}
Diabetic rats + 2.5 mg/kg body weight of glibenclamide	11.04 ± 0.6^{a}	10.30 ± 0.3^{b}	7.28 ± 0.7^{b}	1.69 ± 0.2^{a}	2.06 ± 0.1^{b}	0.23 ± 0.0^{a}
Diabetic rats + 2.5 mg/kg body weight of EACF 5	15.65 ± 0.6^{b}	3.21 ± 0.45^{a}	11.41 ± 1.1^{c}	3.60 ± 0.55^{b}	0.64 ± 0.1^{a}	0.32 ± 0.1^{a}
Diabetic rats + 2.5 mg/kg body weight of EACF7	17.20 ± 0.8^{c}	3.95 ± 0.4^{a}	12.25 ± 1.3^{c}	4.16 ± 0.7^{b}	0.79 ± 0.1^{a}	0.35 ± 0.1^{a}

Values are means of ten replicates ± SEM.

Values with different superscripts are significantly different from each other ($p < 0.05$)

Where EACF = ethyl acetate-partitioned chromatographic fraction

Table 12: Antioxidant Enzymes and Malondialdehyde Levels of Alloxan-induced Diabetic Rats Orally Administered Chromatographic Fractions obtained from Ethyl Acetate-partitioned Fraction from *Chrysophyllum albidum* Stem Bark

Treatment Group	Superoxide Dismutase (unit/mg protein)	Catalase (unit/mg protein)	Malondialdehyde (nmol/L)
Non-diabetic rats + 1mL of distilled water	4.924 ± 0.68^{c}	334.216 ± 37.02^{d}	2.062 ± 0.77^{a}
Diabetic rats + 1mL of distilled water	0.764 ± 0.19^{a}	123.183 ± 25.08^{a}	3.763 ± 0.48^{b}
Diabetic rats + 2.5 mg/kg body weight of glibenclamide	2.517 ± 0.36^{b}	194.885 ± 23.04^{b}	2.43 ± 0.55^{a}
Diabetic rats + 2.5 mg/kg body weight of EACF 5	2.761 ± 0.36^{b}	266.699 ± 41.22^{c}	1.96 ± 0.45^{a}
Diabetic rats + 2.5 mg/kg body weight of EACF7	4.395 ± 0.26^{c}	346.162 ± 82.08^{d}	2.03 ± 0.16^{a}

Values are means of ten replicates ± SEM.

Values with different superscripts are significantly different from each other ($p < 0.05$)

Where EACF = ethyl acetate-partitioned chromatographic fraction

4.7 Pancreas Histology of Alloxan-Induced Diabetic Rats Orally Administered Aqueous and Chromatographic Fractions of Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark

Histological examination revealed distorted histo-architecture of the pancreas of the distilled water treated diabetic rats. The parenchymatous portion of acini and islet portions were completely obliterated, loose connective tissue and adipose tissue seen and are highly infiltrated by inflammatory cells (Plate 3a). Treatment with 25 mg/kg body weight of the aqueous extract of *Chrysophyllum albidum* stem bark showed normal histo-architecture of the pancreas. Furthermore, the pancreas of the rats administered 50, 100 and 200 mg/kg body weight of the aqueous extract of *Chrysophyllum albidum* stem bark revealed distorted histo-architecture of the pancreas while the pancreas of the glibenclamide treated rats revealed distorted histo-architecture of the pancreas with severe necrosis, loose adipose and connective tissues seen (Plate 3a).

The cross section of the pancreas of the distilled water treated diabetic rats revealed distorted histo-architecture of the pancreas (Plate 3b). The pancreas of the glibenclamide treated diabetic rats revealed normal histo-achitecture with mild congestion of the blood vessels. Treatment with F5 revealed distorted histo-architecture with necrosis and severe congestion of the blood vessels while treatment with F7 revealed normal histo-architecture with no visible lesion (plate 3b).

4.8 Toxicological Assessment of the Chromatographic Fractions

4.8.1 Effect of Chromatographic Fractions of Ethyl Acetate-partitioned Fraction from *Chrysophyllum albidum* Stem Bark on Liver and Kidney Function Indices

Administered of F5 significantly ($p < 0.05$) increased the serum urea and creatinine concentrations and decreased the serum electrolytes of the rats. Furthermore, serum albumin, direct bilirubin, total bilirubin and creatinine urea ratio were not significantly altered by both the F5 and F7 whereas F7 did not significantly alter the concentration of urea, creatinine and serum electrolyte (Table 13).

4.8.2 Effect of Chromatographic Fractions of Ethyl Acetate-partitioned Fraction from *Chrysophyllum albidum* Stem Bark on Activity of Some Marker Enzymes

Administration of both the F5 and F7 did not significantly alter the alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities in the liver, kidney and serum as well as serum AST/ALT ratio (Table 14). Furthermore, the liver and kidney activity of aspartate aminotransferase (AST) were not significantly altered by both the F5 and F7 whereas the serum activity of AST significantly decreased by the fractions.

4.8.3 HPLC fingerprinting of the Antidiabetic Constituents in the Chromatographic Fraction F7 Obtained from Ethyl Acetate-partition Fraction of *Chrysophyllum albidum* stem bark

Figure 34 shows the HPLC fingerprint of F7 obtained from ethyl acetate-partitioned fraction of *Chrysophyllum albidum* stem bark. HPLC analysis of the F7 revealed eight peaks (compounds). Among the chemical constituents identified, stigmasterol had the highest peak with a retention time of 1.300 minutes whereas eleagnine had the lowest peak with retention time of 1.066 (minutes) (Table 14).

Table 13: Some Functional Indices of Non-diabetic Rats Orally Administered Chromatographic Fractions obtained from Aqueous Extract of *Chrysophyllum Albidum* Stem Bark

Treatment Group	Urea (mg/dl)	Creatinine (mg/dl)	Creatinine/ Urea	Uric acid (mg/dl)	Albumin (mg/dl)	Bilirubin (mg/dl)	
						Total	Direct
Control	43.80±2.20[a]	0.19±0.02[a]	0.01±0.00 [a]	24.12±1.37 [a]	6.80±0.80[a]	0.79±0.12[a]	0.8 ±0.14[a]
Rats + 2.5 mg/kg body weight of EACF 5	49.35±4.55[b]	0.48±0.12[b]	0.01±0.00 [a]	22.85±1.49 [a]	6.74±1.45[a]	0.68±0.06[a]	0.80±0.23[a]
Rats + 2.5 mg/kg body weight of EACF7	43.34±2.04[a]	0.16±0.03[a]	0.01±0.00 [a]	23.18±1.04 [a]	7.28±1.23[a]	0.95±0.05[b]	1.09±0.36[a]

Values are means of ten replicates ± SEM.

Values with different superscripts are significantly different from each other ($p < 0.05$)

Where EACF = ethyl acetate-partitioned chromatographic fraction

Table 14: Serum Electrolyte of Non-diabetic Rats Orally Administered Chromatographic Fractions obtained from Aqueous Extract of *Chrysophyllum albidum* Stem Bark

Treatment Group	Sodium ion (mmol/L)	Potassium ion (mmol/L)	Chloride ion (mmol/L)	Carbonate ion (mmol/L)	Phosphate ion (mmol/L)
Control	13.11±2.20[b]	8.24±0.94[b]	10.01±0.80[b]	6.16±1.36[b]	7.00±0.91[b]
Rats + 2.5 mg/kg body weight of EACF 5	8.95±1.25[a]	6.88±0.90[a]	6.71±0.00[a]	2.85±1.49[a]	3.01±1.22[a]
Rats + 2.5 mg/kg body weight of EACF7	13.34±1.04[b]	8.01±0.11[b]	10.14±1.14[b]	5.87.18±1.94[b]	7.188±1.03[b]

Values are means of ten replicates ± SEM.

Values with different superscripts are significantly different from each other ($p < 0.05$)

Where EACF = ethyl acetate-partitioned chromatographic fraction

Table 15: Effect of Chromatographic Fractions from Ethyl Acetate-partitioned Fraction of *Chrysophyllum albidum* Stem Bark on Activity of Some Marker Enzymes

Treatment Group	ALP			AST			ALT			AST/ALT
	Liver	Kidney	Serum	Liver	Kidney	Serum	Liver	Kidney	Serum	Serum
Control	475.03±33.88 [a]	172.58±19.35 [a]	218.21±13.40 [a]	86.08±2.29 [a]	82.62±12.28 [a]	153.22±25.08 [a]	221.18±40.84 [a]	251.49±15.68 [a]	218.05±7.61 [b]	2.64±0.62 [a]
Rats + 2.5 mg/kg body weight of EACF 5	459.61±42.95 [a]	156.71±14.28 [a]	206.22±28.52 [a]	82.29±3.02 [a]	75.21±4.70 [a]	134.68±28.77 [a]	263.16±28.88 [a]	220.65±31.68 [a]	196.72±18.43 [a]	2.62±1.80 [a]
Rats + 2.5 mg/kg body weight of EACF7	439.17±40.78 [a]	168.20±34.39 [a]	219.51±4.78 [a]	95.80±18.11 [a]	72.05±1.98 [a]	138.71±29.88 [a]	244.91±62.33 [a]	269.74±52.80 [a]	186.19±7.64 [a]	2.58±3.86 [a]

Values are means of ten replicates ± SEM.

Values with different superscripts are significantly different from each other ($p < 0.05$)

Where EACF = ethyl acetate-partitioned chromatographic fraction

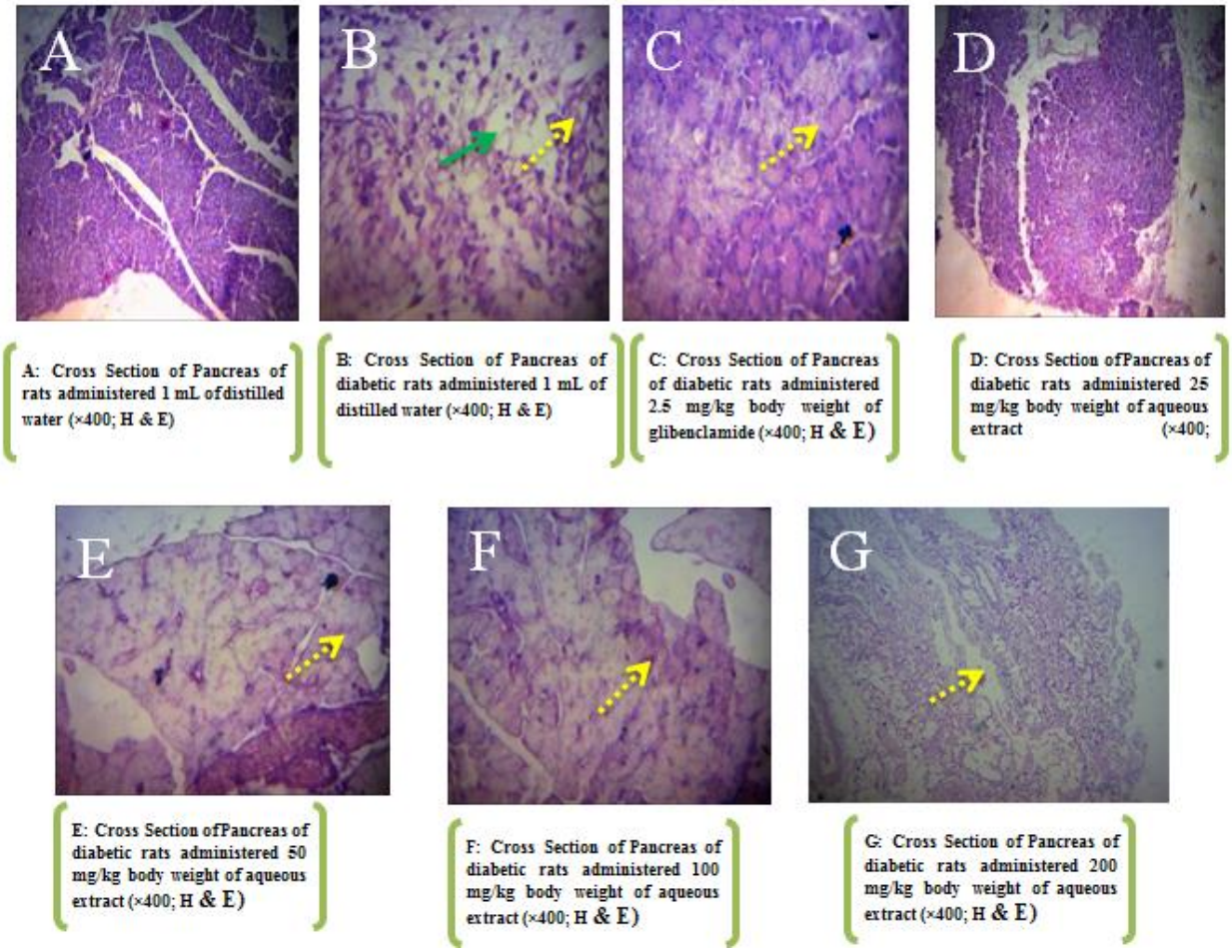


Plate 3a: Cross Section of the Pancreas Administered Aqueous Extract of Chrysophyllum albidum Stem Bark

(A) Normal histo-architecture of the pancreas with no visible lesion

(B) Distorted histo-architecture of the pancreas of the distilled water treated diabetic rats. The parenchymatous portion of acini and islet portions were completely obliterated, loose connective tissue and adipose tissue seen and are highly infiltrated by inflammatory cells

(C) Distorted histo-architecture of the pancreas with severe necrosis, loose adipose and connective tissues seen

(D) Normal histo-architecture of the pancreas with no visible lesion

(E) Distorted histo-architecture of the pancreas with severe necrosis, loose adipose and connective tissues seen

(F) Distorted histo-architecture of the pancreas with severe necrosis, loose adipose and connective tissues seen

(G) Distorted histo-architecture of the pancreas with severe necrosis, loose adipose and connective tissues seen

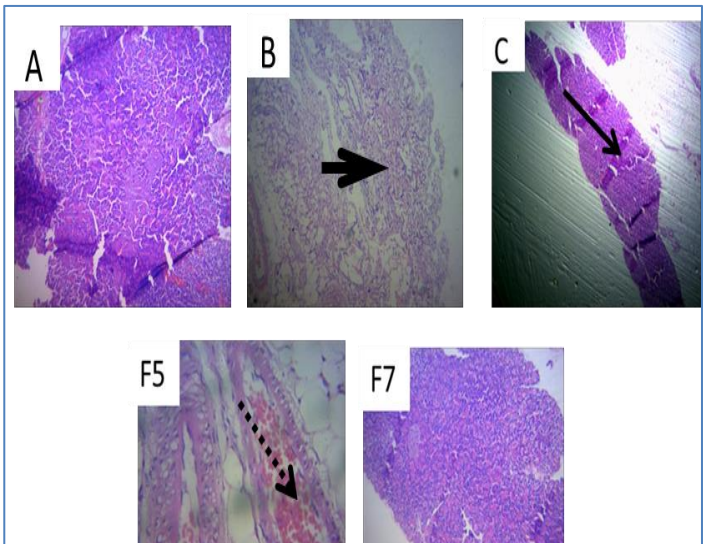


Plate 3b: Cross Section of the Pancreas Administered Chromatographic Fractions from Ethyl Acetate-partitioned Extract of Chrysophyllum albidum Stem Bark

(A) Normal histo-architecture of the pancreas with no visible lesion
(B) Degenerated pancreas with distorted histo-architecture
(C) Normal histo-architecture of the pancreas with with mild congestion of the blood vessels

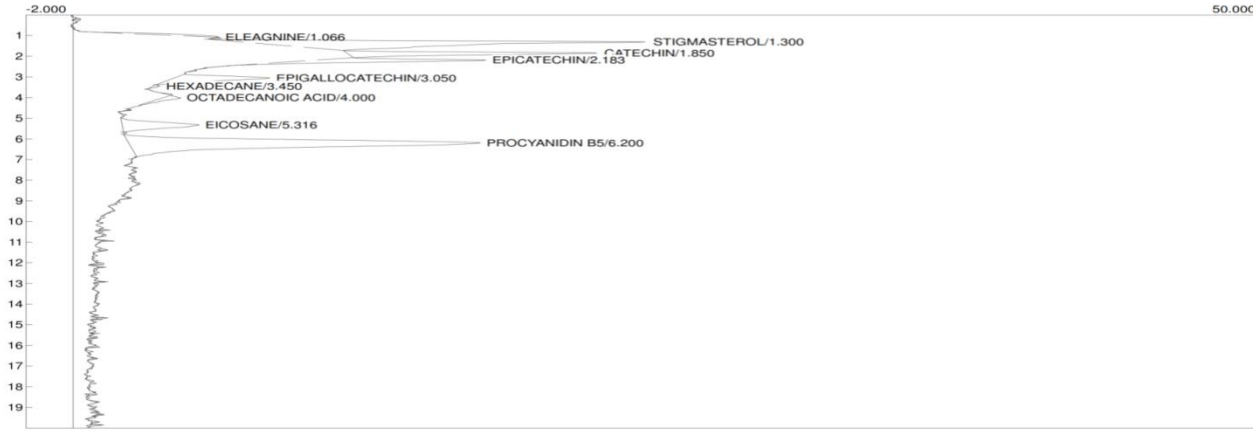
(F5) Distorted histo-architecture with necrosis and severe congestion of the blood vessels

(F7) Normal histo-architecture of the pancreas with no visible lesion

Lab name: Bato Chemical Lab. Ltd
Client: ABDULAZEEZ P. Extract
Client ID: ABDUL
Method: HPLC with UV Detector
Description: CHANNEL 1
Column: uBondapak C18
Carrier: Methanol/Water; 20/80
Data file: ABDULAZEEZ HPLC , CHRYSOPHYLLUM ALBIDUM PLANT EXTRACT . 2912Y17.CHR ()
Sample: Chrysoph.Albidum Extract
Comments: Reconstituted Plant Extract was extracted with Acetonitrile/Methanol , the extract made up in 25ml Standard Flask. 10ul injected @ 1ml/min flow rate.

Temperature program:

Init temp	Hold	Ramp	Final temp



Component	Retention	Area	Height	External	Units
ELEAGNINE	1.066	14.5060	0.358	0.0000	%
STIGMASTEROL	1.300	226.6610	17.205	22.6661	ppm
CATECHIN	1.850	95.5140	10.606	9.5514	ppm
EPICATECHIN	2.183	73.1910	7.177	7.3191	ppm
EPIGALLOCATECHIN	3.050	58.0000	4.039	5.8000	ppm
HEXADECANE	3.450	1.7680	0.284	0.1768	ppm
OCTADECANOIC ACID	4.000	12.2710	0.711	1.2271	ppm
EICOSANE	5.316	62.1060	3.253	0.0000	
PROCYANIDIN B5	6.200	356.5300	14.862	0.0000	
		900.5470		46.7405	

Figure 34: HPLC Fingerprinting of the Constituents of the Chromatographic Fraction (F7) obtained from Ethyl Acetate-partition Fraction of *Chrysophyllum albidum* Stem Bark

Table 16: Chemical Constituents of the Chromatographic Fraction (F7) obtained from Ethyl Acetate-partitioned Fraction from *Chrysophyllum albidum* Stem Bark

Compounds	Retention Time (min)	Concentration (ppm)
Eleagnine	1.066	1.450
Stigmasterol	1.300	2.267
Catechin	1.850	9.551
Epicatechin	2.183	7.319
Epigallocatechin	3.050	5.800
Hexadecane	3.450	0.177
Octadecanoic acid	4.000	1.227
Eicosane	5.316	6.210
Procyanidin B5	6.200	3.655

CHAPTER FIVE

5.0 *In Vivo* Ant-diabetic Study of Aqueous Extract of *Chrysophyllum albidum* Stem Bark

5.1 Blood Glucose

Alloxan is one of the chemicals commonly employed to induce type 1 diabetes mellitus (Srinivasan and Ramarao, 2007). It uptake by the beta cell of the pancreas due to its structural similarity to glucose, lead to the generation of free radicals that results in necrosis and eventually cell death (Lenzen, 2008). Necrosis or death of the beta cells reduced insulin production that impaired glucose utilization, alter protein and lipid metabolism (Ozougwu *et al*., 2013). Mobilization of fuel from adipocytes and muscle cells further reduce glucose uptake and suppress its metabolism in the muscle and the adipocytes which further exacerbate already highy glucose level (Ozougwu *et al*., 2013), and hence diabetes. The standard antidiabetic drug used, glibenclamide, is a sulfonylurea that act by inhibiting the ATP-sensitive potassium channels in the pancreas which leads to the depolarization of the cells and insulin exudation (Luzi and Pozza, 1997). The increase in glucose level of distilled water treated diabetic rats may be attributed to reduction or lack of insulin production by the beta cell of the pancreas which was exacerbated by mobilization of fuel from the muscle and the fat cells. The reduction of the elevated blood glucose level of the diabetic rats after extract administration suggests that the extract might be acting by uptimizing glucose utilization and insulin excretion (Uma *et al.*, 2014). This is in line with the findings of Mnonopi *et al* (2012), Ozougwu *et al* (2013) and Yakubu and Ogunro (2014) that attributed the increase in fasting blood glucose in diabetic animals to mobilization of fuel from other sources due to lack of insulin.

5.2 Glucose and Glycogen

Glucose is a necessity in a wide range of biological reactions and its excess is toxic. Thus, cellular glucose consumption is tightly regulated to prevent lasting hyperglycemia and conserve sugar in the form of glycogen for later use (Gusarov and Nudler, 2018). As glycogen can yield glucose-6-phosphate when the need arise. It serves as a temporal buffer for glucose overflow, which is continuously degraded and resynthesized depending on immediate energy requirements. The increase in hepatic glucose with decreased glycogen content in the distilled water treated diabetic rats might be due to increase in hepatic glucose output stimulated by declined insulin ooze (Xiao *et al.,* 2017). Oral administrations of aqueous extract of Chrysophyllum albidum stem bark significantly reduced the exacerbated hepatic glucose level and increase its glycogen content. This might be due to inhibition of gluconeogenesis, glycogenolysis and activation of glycolysis, glycogenesis and lipogenesis (Satoh, 2018).

5.3 Carbohydrate Metabolizing Enzymes

Liver plays a distinctive role in adjusting carbohydrate metabolism by regulating glucose levels through a closely regulated collection of enzymes (Raddatz and Ramadori, 2007). The activity of these enzymes is altered in diabetes (Ramesh *et al.,* 2017). Various apparatuses have been linked to the antidiabetic activities of medicinal plants including peripheral utilization of glucose, increased production of hepatic glycogen, inhibition of carbohydrate metabolizing enzymes, stimulation of pancreatic insulin release, and inhibition of hepatic glucose production (Krishnasamy *et al.,* 2016).

Glucokinase, a rate limiting enzyme commonly stimulated by insulin, facilitates glucose utilization by converting glucose to glucose-6-phosphate in the liver (Lee *et al*., 2014; Eze *et al.,* 2016). The decrease in the activity of this enzyme in the distilled water treated diabetic treated rats might be due to lack of insulin. This signals the release of glucose

from the stores which further exacerbate hyperglycaemia (Agius, 2008 Iynedjian, 2009). The increase in glucokinase activity of the extract treated diabetic rats might be due to activation of glycolysis (Eze *et al.,* 2016). This suggests that the extract demonstrated its hyperglycaemic effects by enhancing glucose utilization through glycolysis, glycogenesis, lipogenesis (Eze *et al.,* 2016). This finding has been substantiated by other investigators (Eze *et al.,* 2016) who attributed the increase in glucokinase activity to enhance glucose utilization. Glucose-6-phosphate dehydrogenase (G6PDH), a rate limiting enzyme in the pentose phosphate pathway that speeds up the oxidation of glucose-6-phosphate to 6-phospho gluconate and at the same time reducing NADP+ to generate NADPH. NADPH thus formed is essential in suppressing oxidative stress (Jain *et al.,* 2004). The decrease in the G6PDH activity of the distilled water treated diabetic rats might be due to hyperglycemia that has been linked to decreased activity of G6PDH (Ramash *et al.,* 2017). Oral administration of the extract significantly increases the activity of the enzyme. This suggests that the extract might suppress oxidative stress associated with diabetes and enhance glucose utilization via pentose phosphate pathway. The significant decrease in alpha amylase activity in the tissues studied compared to distilled water treated diabetic rats, signified inhibition of alpha amylase. This delays glucose absorption and moderates postprandial blood sugar level which plays a central role in development and progression of diabetic complications (Ortiz-Andrade *et al*., 2007, Sabiu and Ashafa, 2016). The antidiabetic mechanism of action of *Chrysophyllum* albidum stem bark might be through increase utilization of glucose by peripheral tissues, increased synthesis of hepatic glycogen, inhibition of alpha amylase, regeneration of the pancreas and inhibition of hepatic glucose production (Krishnasamy *et al.,* 2016).

5.4 Selected Biomarkers

The elimination of urea and creatinine from the plasma is the primary function of glomerulus and is normally use for the assessment of renal competence (Saba *et al.,* 2007).

The increase in urea concentration of the distilled water treated diabetic rats might be due to deamination of glucogenic amino acids from gluconeogenesis, increase muscle proteolysis and reduced protein synthesis (Sunmonu and Afolayan, 2013). Creatinine, a metabolite of muscle creatine usually has constant concentration which becomes elevated when renal function is impaired. The increase in creatinine level of the distilled water treated diabetic rats might be due to impairment of renal function resulting from hyperglycaemia. Electrolyte imbalance is one of the contributors of diabetes symptoms including polyuria, polydipsia, polyphagia, weight loss, and blurry vision which may lead to impediments (Maniatis *et al.*, 2005). The disturbance in the electrolyte of distilled water treated diabetic rats might be due to hyperglycemia which sets the internal environment for osmotic diuresis while causing a dilution effect on electrolyte concentrations. The osmotic effect of glucose results in dwindled circulating blood volume and fluid shift from the intracellular spaces causing cellular thirst (Khanduker *et al.,* 2018). The decrease in the level of urea, creatinine and restoration of electrolytes to near normal suggest that the extract might prevent or ameliorate hyperglycaemia-induced renal dysfunction associated with diabetes mellitus. This is in line with the finding of the previous investigators (Yakubu and Ogunro, 2014; Sunmonu and Afolayan, 2013 and Ibrahim *et al.,* 2018) that attributed the reduction of the alloxan-induced increased biochemical parameters to ameliorative potential of the plant extracts.

Assessment of haematological parameters could be used to divulge the harmful effect of foreign compounds including plant extracts on the blood components of animals; and to determine possible shifts in the levels of biomolecules such as enzymes, metabolic products, normal functioning and histomorphology of the organs (Magalhães, 2008). The decreased erythrocytic indices (red blood cell count, haemoglobin and pack cell volume) in the distilled water treated diabetic rats might be due to hyperglycaemia. The osmotic effect of glucose which results not only in dwindled circulating blood volume and fluid shift from the

intracellular spaces causing cellular dryness, but also in glycosylation of haemoglobin (Yakubu and Ogunro, 2014; Khanduker *et al.,* 2018). The reversal of the decreased erythrocytic indices might be due to recovery from alloxan assault and capable of ameliorating erythrocytic disorder associated with diabetes as evident in this study.

5.5 *In vitro* Study

Inhibition of α-amylase and α-glucosidase by the extract is an indication that the extract might produce a postprandial antihyperglycemic effect by suppressing carbohydrate metabolism and the consequent glucose release from the lumen of small intestine (Shalev, 1999; Matsui *et al,* 2007). Inhibition of the two enzymes *in vitro* by the extract can be linked with enzyme inhibition *in vivo,* which is capable of subsiding glucose entering portal vein from the gut or glucose production from starch (Matsui *et al.,* 2001). This finding is consistent with previous investigators (El-Beshbishy and Bahashwan 2012; Ezeani *et al*., 2017) that attributed the inhibition of the enzymes *in vitro* to suppression of glucose metabolism *in vivo*.

The relationship between generation of free radicals and the pathogenesis of DM and its complications has been demonstrated (Ezeani *et al.*, 2017). In the present study, the aqueous extract of *Chrysophyllum albidum* and its solvent partitioned fractions exhibited significant anti-radical activities as shown by its IC_{50} values when compared with the reference antioxidant used. The closeness of the r^2 values to 1.0 is not only indicative of either exactness or accurate submissions for the assays, but also informative of the ability of the extracts to stall free radicals chain reactions associated with diabetes complications. This is consistent with previous *in vitro* studies (Apostolidis *et al*., 2007; Sabiu *et al*., 2016b).

5.6 Antidiabetic Study of Ethyl Acetate-partitioned Fraction

The increase in FBG of the alloxan-induced diabetic rats might be due to exacerbation of hyperglycemia resulting from dearth of insulin (Ozougwu *et al*., 2013). The reduction of

the elevated blood glucose level of the diabetic rats after the administration of the ethyl acetate-partitioned fractions suggests that the extract might be acting by enhancing insulin secretion and improving glucose utilization (Uma *et al.*, 2014). The decrease in hepatic glucose with increase in hepatic glycogen content following administration of the ethyl acetate-partitioned fractions might be due to inhibition of gluconeogenesis, glycogenolysis and activation of glycolysis, glycogenesis and lipogenesis (Satoh, 2018). The increase in G6PDH activity of the ethyl acetate-partitioned treated rats suggests enhanced glucose utilization via pentose phosphate pathway (Ramash *et al.*, 2017). The significant decrease in alpha amylase activity in the tissues studied compared to distilled water treated diabetic rats, signified inhibition of alpha amylase. This delays glucose absorption and moderates postprandial blood sugar level which plays a central role in development and progression of diabetic complications (Ortiz-Andrade *et al.*, 2007, Sabiu and Ashafa, 2016). The significant increase in cholesterol, triglycerides with decrease in HDL level in the distilled water treated diabetic rats might be due to abnormality in insulin secretion which triggered the mobilization of free fatty acid from the fat deposit (Ahmed, 2001). Administration of the ethyl acetate-partitioned fractions significantly decreased cholesterol, triglycerides and increased HDL level. This might be due to release of insulin from the pancreas thereby activating lipoprotein lipase which hydrolyses triglycerides and inhibits the release of free fatty acids from the fat stores (Kehlenbrink *et al.*, 2012). The decrease in the antioxidant enzyme system with increase in malondialdehyde (MDA) level of the distilled water treated diabetic rats in this study might be due to oxidative strain and lipid peroxidation correspondingly. Administration of the fractions of the extract however amplified the antioxidant enzyme activities and reduced the MDA level. This suggests that the extract might boost the antioxidant system and impedes lipid peroxidation (Tiwari et al., 2013).

5.7 *In vivo* Antidiabetic Study of Ethyl Acetate Chromatographic Fractions

5.7.1 Blood Glucose and Body Weight

In this study, the significant upsurge in fasting blood glucose level of the distilled water treated diabetic rats might be due to annihilation of the insulin producing beta cells of the pancreas by alloxan. The resulting hyperglycaemia might be the summation of glucose fluxes from gluconeogenesis, glycogenolysis, lipogenolysis and other pathways resulting from lack of insulin production (Petersen *et al*., 2017). Administration of F5 and F7 however, abridged the fasting blood glucose level of the alloxan-induced diabetic rats. This effect might be owing to the presence of phytochemicals (tannin, saponin, flavonoids, phenols, and alkaloids) previously reported by Okoli and Okere (2010). These phytochemicals might be acting singly or in synergy with another and might be responsible for the hypoglycaemic effect of the chromatographic fractions observed in this study. This suggests that the fractions might be acting by improving glucose utilization through the activation of glycolysis, glycogenesis and lipogenesis.

The substantial fall in body weight of the distilled water treated diabetic rats when compared with distilled water treated non-diabetic rats might be due to increased degradation of structural proteins as a result of damage to the intracellular signaling responsible for maintaining the balance between the synthesis and breakdown (Hulmi *et al*., 2011; Newsholme *et al*., 2011). The significant increase in the body weight of the F5 and F7 treated groups suggest that the fractions might have bioactive potency similar to insulin growth factor-1 which increases protein synthesis in diabetes to restore muscle wasting through the activation of Akt/mTOR pathway (Zhang *et al*., 2014).

5.7.2 Feed and Water Intake

Glucose is an osmotic diuretic and an increase in renal loss of glucose is accompanied by loss of water and electrolyte. This leads to fall in intracellular water volume that results in

the triggering of the thirst mechanism (polydipsia). The undesirable caloric balance, which results from the tissue breakdown leads to an increase in appetite and food intake known as polyphagia (Raju and Raju, 2010). The increase in feed and water intake of the distilled water treated diabetic rats, observed in this study, might be due to the aggravation of tissue catabolism which results in increase glucose output accompanied by an increased renal loss of water that stimulates the osmoreceptor of the thirst centre of the brain to crave for water. Interestingly, oral administration of F5 and F7 significantly reduced the trend. This suggests that the extract prevents polydipsia and polyphagia associated with diabetes. This finding supports the report of Kim *et al* (2006).

5.7.3 Oral Glucose Tolerant Test

Oral glucose tolerant test (OGTT) is very important in screening and diagnosis of pre-diabetes or diabetes as it measures the body's ability to use glucose (Agwaya and Nandutu, 2016; Pari and Chandramohan, 2017). In this study, the significant increase in the blood glucose level of the alloxan-induced diabetics at 30 minutes which persistent for 120 min following OGTT suggests poor clearance of the glucose from the system. In contrast, the return of F7 to basal glucose level among the treatment groups after 120 minutes implied that only F7 enhance glucose utilization and hence improve glucose tolerance in diabetic rats. Similar observations were reported by Sharma *et al* (2011) and Agwaya and Nandutu (2016).

5.7.4 Glucose and Glycogen

While glucose serves as the currency for biological system, glycogen remained the major glucose storage cache that is critical for energy homeostasis (Raththagala *et al.*, 2015). The production of glucose during fasting and its storage postprandially by the liver is dysregulated in diabetes mellitus (Petersen *et al.*, 2017). The upsurge in hepatic glucose with decreased liver glycogen content in the alloxan-induced diabetic rats might be due to decline in insulin level caused by the destruction of the insulin producing beta cells of the

pancreas as evident in this study. Oral administration of the chromatographic fractions of the extract however significantly reduced the hepatic glucose with increased glycogen level. This might be due to stimulation for increase production of insulin from the residual beta cells of the pancreas by the fractions of the extract. This suggests that the fractions of the extract increased glucose utilization by increasing the glucokinase activity (Grimsby *et al.,* 2003; Pfefferkorn, 2013). This might in turn reduced the hepatic glucose production and increase the use of glucose-6-phosphate formed for glycogen synthesis.

5.7.5 Serum Insulin

Insulin is central to the maintenance of glucose homeostasis in various tissues including liver, skeletal muscle and the fat cells (Bogan, 2012; Newsholme *et al*., 2014). It facilitates the uptake of glucose in the circulation through translocation of glut4 transporter (Satoh, 2018). The significant decrease in insulin level of the alloxan induced diabetic rats when compared with the distilled water treated non-diabetic rats might be due to destruction of the insulin producing beta cells of the pancreas resulting from alloxan administration (Lenzen, 2008). This might reduce the expression of a number of proteins such as glucokinase and the GLUT4 glucose transporter gene required for normal response to insulin in liver and adipose tissue, respectively (Ozougwu *et al.,* 2013). Administration of the chromatographic fractions of the extract however increased the insulin level of the chromatographic fractions treated diabetic rats. This suggests that the fractions of the extract might be acting by regenerating the beta cells of the pancreas and thereby improved insulin secretion. This will in turn activate the proteins involved in glucose metabolism (glucokinase and GLUT4 glucose transporter protein) and lipid metabolism (lipoprotein lipase and fatty acid transporter protein), as well as energy balance (Moses, 2010; Raju and Raju, 2010; Atangwho *et al*., 2014; Unnikrishnan *et al*., 2014; Kumar *et al*., 2015; Chaudhury *et al*., 2017). All these lead to inhibition of gluconeogenesis and increased the conversion of

glucose to glycogen for future use (Smith and Turner, 2017). This is in line with the findings of the previous researchers that reported the improvement in insulin secretion by the pancreas of diabetic animals after extract treatment (Choi *et al*., 2004; Park *et al*., 2012).

5.7.6 Carbohydrate Metabolizing Enzymes

Glucokinase is glucose rate-controlling enzyme for hepatic glucose clearance and glycogen synthesis and is the most sensitive indicator of the glycolytic pathway in diabetes (Eze *et al.*, 2016). Glucokinase aids in glucose utilization by transforming glucose to glucose-6-phosphate in the liver (Lee *et al*., 2014). Activation of glucokinase by glucose and insulin promotes glycogen synthesis in the liver (Zelent *et al.*, 2011, Nakamura and Terauchi, 2015). The decrease in the hepatic glucokinase activity of the distilled water treated diabetic rats observed in this study might be due to reduced insulin secretion by the pancreas. The significant increase in the glucokinase activity of the chromatographic fractions treated diabetic rats might be due to improved glucose utilization through the triggering of glycolysis (Eze *et al.*, 2016). This suggests that the chromatographic fractions of the extract might prevent the derangement of nutrients metabolism associated with diabetes, halt the progression of diabetes, enhance glucose utilization, reduce hepatic glucose production and promote glycogen synthesis (Agius, 2008; Iynedjian, 2009). This discovery has been corroborated by other investigators (Eze *et al.*, 2016).

Gluconeogenesis contributes 50–60 % of the hepatic glucose production in diabetes (Srinivasan *et al.* 2014). Glucose-6-phosphatase and fructose-1,6-bisphosphatase are fundamental enzymes in modifying this pathway. In this study, the activities of the hepatic gluconeogenic enzymes were significantly increased in the distilled water treated diabetic rats. The increased activities of these enzymes may be due to the initiation of the enzymes known to contribute to the amplified glucose production during diabetes mellitus (Pari and Chandramohan, 2017). However, the significant decrease in the activity of the enzymes after

chromatographic fraction treatment might be due to inhibition of gluconeogenesis by decline in the activities of glucose-6-phosphatase and fructose1, 6-bisphosphatase, thereby reducing the endogenous production of glucose from the hepatocytes. These results are in agreement with the previous investigators (Jung *et al*., 2004; Pari and Chandramohan, 2017).

Glucose-6-phosphate dehydrogenase is the key regulatory enzyme in the pentose phosphate pathway, which results in the production of ribose-5-phosphate and NADPH (Cappai *et al.* 2011). The significant decrease in hepatic glucose-6-phosphate dehydrogenase activity in the distilled water treated diabetic rats might be due to decrease in glucose metabolism via phosphogluconate oxidation pathway (Prasath and Subramanian, 2011). Oral administration of the chromatographic fractions of the extract significantly increased the activity of the enzyme in the diabetic rats. This suggests that the extract enhanced the glucose utilization by channeling excess glucose into the pentose phosphate pathway (Prasath and Subramanian, 2011). This agrees with the previous researchers (Prasath and Subramanian, 2011) who attributed the increase G6PDH activity to improved glucose utilization via pentose phosphate pathway.

5.7.7 C-reactive Protein

C-reactive protein (CRP), a plasma protein made by the liver, is a sensitive and dynamic systemic indicator of inflammation (Pepys and Hirschfield, 2003). The significant increase in the CRP level of the distilled water treated diabetic rats might be due to inflammation of the pancreas (Mirochnik *et al.*, 2017). This suggests a chronic hyperglycaemia which has been reported to increase the circulating inflammatory biomarkers (Spranger *et al*., 2003). The significant decrease in CRP level of the extract treated diabetic rats suggests that the extract ameliorated inflammation and prevent secondary complications like coronary heart disease via reduction of CRP level. This finding agrees with the report of Mahluji *et al* (2013) and Gargari *et al* (2015).

5.7.8 Glycosylated Haemoglobin

Glycosylated haemoglobin is a reliable index to monitor glucose lowering therapy and long-term glycaemic control (Kasetti *et al.* 2010; Sasikala and Sudhakar, 2015). In diabetic condition, blood glucose rejoins non-enzymatically with hemoglobin and accordingly increased glycosylated hemoglobin concentration (Pamu *et al.* 2015). The substantial rise in the levels of glycosylated haemoglobin in the distilled water treated diabetic rats might be due to formation of non-enzymatic glycosylation of the surplus glucose in circulation. (Sundaram *et al.* 2013). Oral administration of chromatographic fractions of the extract decreased the elevated glycosylated haemoglobin which might be due to tight glycaemic control as glucose level has been reported to be directly related to glycosylated haemoglobin level (Moussa, 2008). This suggests that the extract was able to prevent the autoxidation of glucose, the non-enzymatic and progressive glycation of protein while enhancing glucose flux into pentose phosphate pathway. The finding is in agrees with the finding of the previous investigators (Moussa, 2008 Pamu *et al.* 2015)

5.7.9 Lipid Profile

Dyslipidemia, an abnormal metabolism of lipoproteins is central to poorly managed diabetes (Jacobson *et al.,* 2007) usually characterized by decrease HDL and increase total cholesterol, triglycerides, low density lipoprotein and very low density lipoprotein (Komolafe *et al*., 2009; Lee *et al*., 2014). This ruckus in lipid profile is a major risk factor for coronary heart disease (Raju and Raju, 2010). The significant rise in cholesterol, triglycerides, low density lipoprotein and very low density lipoprotein and atherogenic index with decrease in HDL level in the distilled water treated diabetic rats might be due to abnormality in insulin secretion which triggered the mobilization of free fatty acid from the fat deposit (Ahmed, 2001). Administration of the chromatographic fractions (F5 and F7) however significantly decreased cholesterol, triglycerides, low density lipoprotein and very low density lipoprotein,

atherogenic index and increased HDL level. This might be due to brisk release of insulin from the pancreas activating lipoprotein lipase which hydrolyses triglycerides and inhibits the release of free fatty acids from the fat stores (Kehlenbrink *et al*., 2012). This suggests that the chromatographic fractions of the extract might be preventing dyslipidemia by stimulating fatty acid biosynthesis, increase utilization of glucose and decrease mobilization of free fatty acids from the stores and incorporation of free fatty acids into triglycerides in the liver and adipose tissues by inhibiting the hormone sensitive lipase (Strålfors and Honnor, 1989).

5.7.10 Lipid Peroxidation and Antioxidant Enzymes

The liver plays an essential role in many metabolic processes and as such may be susceptible to oxidative stress and mutilation (Petrus *et al.,* 2011) which has been implicated in diabetes (Giacco and Brownlee, 2010). This is usually accompanied by an increased level of inflammation (Korkmaz and Kolankaya, 2009). Antioxidant agents protect against cellular damage by boosting the antioxidant enzyme capacity of the body (Mohamed *et al.,* 2012). The decrease in the antioxidant enzyme system with increase in malondialdehyde (MDA) level of the distilled water treated diabetic rats in this study might be due to oxidative stress and lipid peroxidation correspondingly. Administration of the fractions of the extracts however boosted the antioxidant enzyme system and reduced the malondialdehyde level. This suggests that the extract improved superoxide dismutase (SOD) concentration to provides first line of defense against ROS mediated cell injury, by catalyzing the proportion of superoxide to molecular oxygen and peroxide (Tiwari et al., 2013) while catalase converts the peroxide formed into water and oxygen. The reduction in the MDA level observed in this study might be due to decreased ROS facilitated by increased antioxidant enzyme activity (Asmat *et al*., 2015). This is in agreement with the finding of Ayepola *et al* (2014), Moussa (2008).

5.8 Histological Studies

Histological examination of the pancreas revealed parenchymatous portion of acini and islet portions completely obliterated with loose connective and adipose tissues. Diabetics administered doses of aqueous and chromatographic fractions of the extract of *C. albidum* stem bark revealed varied levels of pancreas degeneration ranging from mild to severe congestion of the blood vessels. Diabetic rats administered 25 mg/kg body weight (aqueous) and 2.5 mg/kg body weight (chromatographic fraction) of the extract did not alter pancreatic histo-architecture of the rats (Plate 3). Alloxan causes state of insulin-dependent diabetes through its ability to induce reactive oxygen species formation, resulting in the selective necrosis of beta cells. The disruption of the pancreas histo-architecture observed in this study might be due to the generation of free radicals that causes the death of the beta cells (Lenzen, 2008). However, administration of 25mg/kg body weight of the aqueous extract and 2.5 mg/kg body weight of F7 restored the degenerated pancreatic histo-architecture induced by alloxan administration. This suggests that the extract and its fractions (F7) possess antioxidant property which enabled it to combat the free radicals generated by alloxan induction of diabetes and hence the regeneration of the pancreas.

5.9 Toxicological Studies

Kidney helps in sustaining homeostasis of the body by retaining important material and evacuating waste products (James *et al.*, 2010). Its function is usually assessed through a battery of metabolites including urea, uric acid, creatinine and serum electrolytes (Ranjna, 1999). Alteration in the levels of these metabolites signifies renal and osmotic imbalance (Dzoyem *et al.*, 2014). In this study, the non-significant difference in the level of urea, creatinine, creatinine:urea ratio and uric acid of both fractions of the extracts implies non alteration of the renal function. However, the decrease in the serum electrolyte of the F5 treated rats might be due to the inhibition of Na+/K+- ATPase activity (Mohamed 2010).

This suggests that F5 though, did not produce renal dysfunction, it is not as safe as F7 and may damage membrane bilayer if the usage is prolonged.

Albumin and bilirubin are commonly used in addition marker enzymes to access hepatic function (Dzoyem *et al.,* 2014; Kalaiselvi *et al.,* 2015).). Whereas alkaline phosphatase is a primary marker of hepatobiliray integrity (Bhattacharya *et al.,* 2013), alanine and aspartae aminotransferases are mostly used to access hepatic injury (Ramaiah, 2007). Because alanine aminotransferase is more specific than aspartate aminotransferase, AST/ALT ratio is commonly employed as a more specific marker of hepatic injury (Anju and Shah, 2017). Administration of chromatographic fractions of aqueous extract of *Chrysophyllum albidum* stem bark did not significantly alter the ALP, ALT, AST (liver, kidney and serum) and AST/ALT. This suggests that administration of the extract did not alter biological functions of the body.

5.10 HPLC Analysis of the Chromatographic Fraction F7

Medicinal plants are the main source of organic compounds that represent a source for the discovery and development of new brands of pharmacological molecules. Many compounds isolated from plant sources including procyanidins and marubin have been reported to show antidiabetic activity (Firdous, 2014). The phytochemicals identified by the HPLC analysis of chromatographic fraction F7 obtained from ethyl acetate-partitioned fraction of *C. albidum* stem bark, might be responsible for the antidiabetic activity observed in this study.

Procyanidins, isolated from natural sources (Yamashita *et al.,* 2012) possess various beneficial effects for health promotion including the prevention of diabetes mellitus (Kanamoto*et al.,* 2011). The contrivance of its anti-diabetic activity depends on the level of its polymerization. Low degree polymerization procyanidins may promote glucose uptake activity by inducing GLUT4 translocation through the AMPK-dependent pathway in muscle,

while high degree polymerization procyanidins may delay glucose absorption by inhibiting α-glucosidase in small intestine (Yamashita *et al.,* 2012). This suggest that the antidiabetic mechanism of procyanidins identified in the plant might be through the translocation of GLUT4 glucose transporter in the muscle or by impeding the absorption of glucose in the small intestine which has been validated *in vitro* in this study.

Stigmasterol, a member of phytosterol family, widely exists in plant oil and plant-based foods. It has a structure related to cholesterol with no impact on human cholesterol (Abumweis *et al.*, 2008; Li *et al.,* 2015). It has various pharmacological properties (Gabay *et al.,* 2010; Kim *et al.,* 2014; Zielińska *et al.,* 2014; Casal *et al.,* 2016). Wang *et al.*, 2017 concluded that stigmasterol effectively ameliorated the hyperglycemia and hyperlipidemia in KK-Ay mice by enhancing GLUT4 expression and translocation.

The antidiabetic activity of *C. albidum* stem bark might be due the presence of these secondary metabolites acting individually or synergistically.

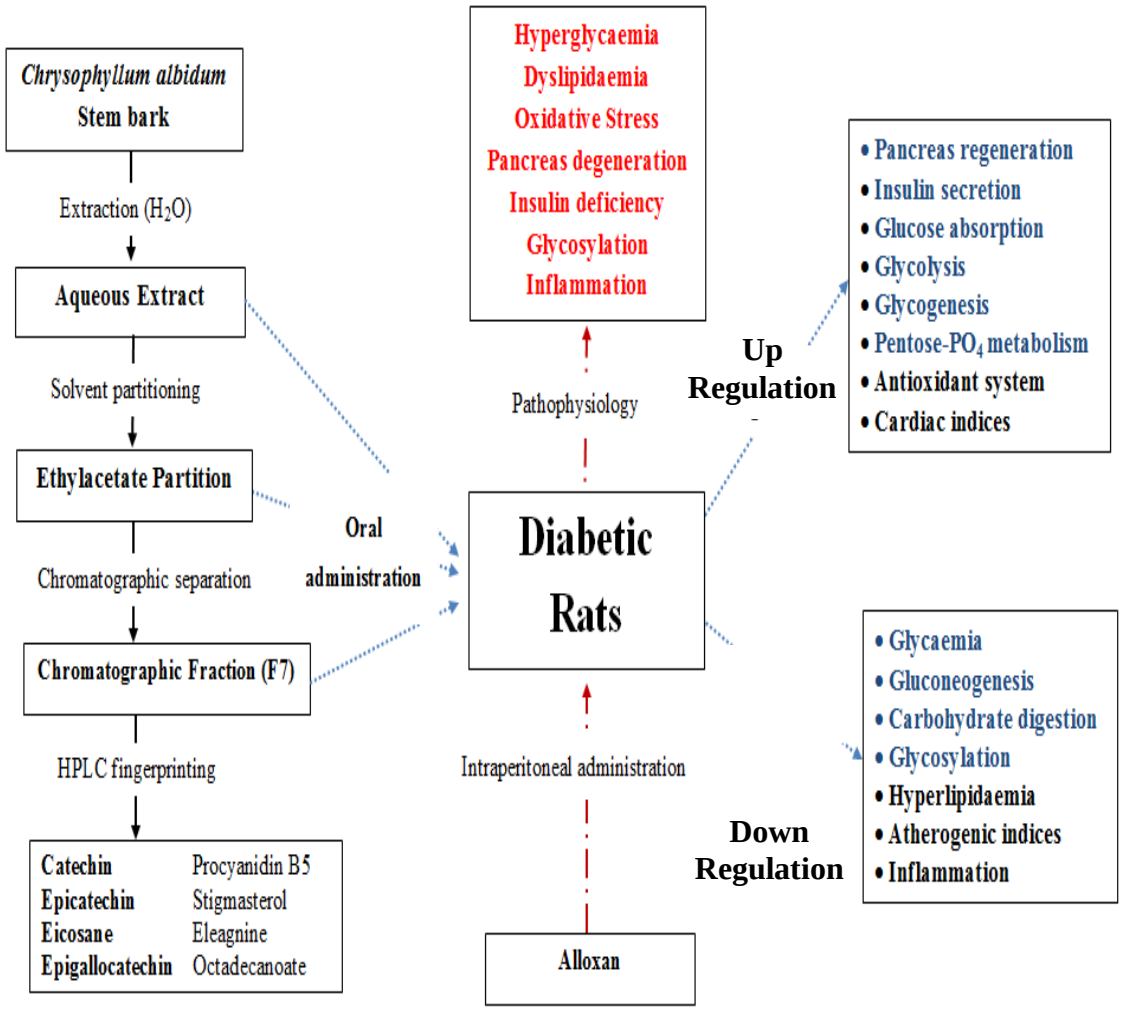


Figure 35: Scheme of the proposed Mechanism of Action of *Chrysophyllum albidum* Stem Bark

FINDINGS

The following findings were made from the results obtained in this study:

1. The aqueous extract of *C. albidum* stem bark exhibited anti- diabetic activity.
2. Ethyl acetate-partitioned fraction exhibited the most anti-diabetic activity among the solvent fractions.
3. Chromatographic fraction (F7) displayed the most profound anti-diabetic activity and ameliorated dyslipidemia and lipid peroxidation associated with diabetes.
4. The likely antidiabetic compounds present in F7 obtained from *C. albidum* stem bark were stigmasterol, Procyanidin B5, catechin and its derivatives.

CONCLUSION

Based on the results obtained from this study, it could be concluded that the aqueous, ethyl acetate-partitioned and the F7 of *C. albidum* stem bark have anti- diabetic activity and ameliorate dyslipidemia and lipid peroxidation associated with diabetes. These effects were optimal at lower doses of 25 and 2.5 mg/kg body weight of the aqueous and ethyl acetate-partitioned fractions respectively. Also, the phytochemicals identified in the chromatographic fraction of the extract might be responsible for the antidiabetic and dyslipidemia and lipid peroxidation ameliorative effects observed in this study. Finally the extract is safe at 25 and 2.5 mg/kg body weight of the aqueous and ethyl acetate-partitioned fractions respectively.

RECOMMENDATION

Recommendation based on the study

Principles identified in the *Chrysopyllum albidum* stem bark may be explored in the formulation of antidiabetic drug.

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APPENDIX I

PREPARATION OF REAGENTS AND BUFFERS

(a) Preparation of Alloxan

5% solution of alloxan monohydrate

2.5g of alloxan was dissolved in small quantity of sterile distilled water and made up to 50ml with distilled water.

Administration of 120mg/kg of alloxan monohydrate

1000g body weight of requires 120mg (120 x 10^{-3}g) of alloxan

200g rat will required 30mg of alloxan

Xg body weight of rat will be injected 0.12 Xmg

5g of alloxan is dissolved in 100ml of solution

0.05g (50mg) will be in every 1ml

Then, 0.12 Xmg will be injected with 0.0030 Xml

If a 200g rat; X = 200

0.0030 Xml = 0.60ml

A rat of 200g body weight will be injected with 0.60 ml of 5% alloxan monohydrate solution.

Reagent for Histopathology

10% Buffered Formalin

9.0 g of sodium chloride was dissolved in distilled water to make 500 ml solution. 100 ml of formalin was slowly added with continuous stirring and made up to 1 litre with distilled water.

Reagents for Protein Determination

Biuret Reagent

1.5 g of copper sulphate ($CuS0_4.5H_20$) and 6.0 g of sodium potassium tartarate ($NaK.C_4H_4O_6.4H_2O$) were dissolved in distilled water to make 500 ml solution (solution A).

3 g of sodium hydroxide (NaOH) was dissolved in distilled water to make 300 ml Solution B was then added slowly to solution A with continuous stirring and the resulting solution transferred to a one-litre standard flask and the volume made up to the marked level with distilled water. The solution was stored in a polythene bottle.

Reagent for Phosphatases

(i) Alkaline Phosphatase (ALP)

Carbonate Buffer (0.1M, pH 10.1)

(a) 2.10 g of sodium bicarbonate ($NaHCO_3$) was dissolved in 250 ml distilled water.

(b) 7.2035 of sodium carbonate ($Na_2CO_3.H_2O$) was dissolved in distilled water to make 250ml solution .Solution A was added to Solution B with continuous stirring until PH of the mixture comes to 10.1

Para-nitro Phenyl Phosphate (10mM)

36.7 mg paranitro phenyl orthophosphate was dissolved in distilled water and made up to 100ml in a 100ml standard volumetric flask.

Sodium Hydroxide (1N)

40 g of sodium hydroxide pellet was dissolved in small quantity of distilled water and made up to 1 litre in a one-litre standard volumetric flask.

Magnesium Sulphate (0.1M)

2.464 g of magnesium sulphate ($MgSO_4.7H_2O$) was dissolved in distilled water to make 100 ml solution.

Reagents for aspartate transaminase (AST) and alanine transaminase (ALT)

0.4 N Sodium Hydroxide

16 g of sodium hydroxide was dissolved with small quantity of distilled water in a beaker and made up to 1 litre in a one litre volumetric flask.

Preparation of 4% Copper Acetate Solution

4g of copper acetate in 100ml of distilled water.

Preparation of 6M HCl

$$\text{volume (mls)} = \frac{\text{\% purity x 100}}{\text{M.M x specific gravity}}$$

51.11mls of concentrated HCl was added to distilled waer in a 100ml standard flask and make up to the mark with distilled water

APPENDIX II

TABLES

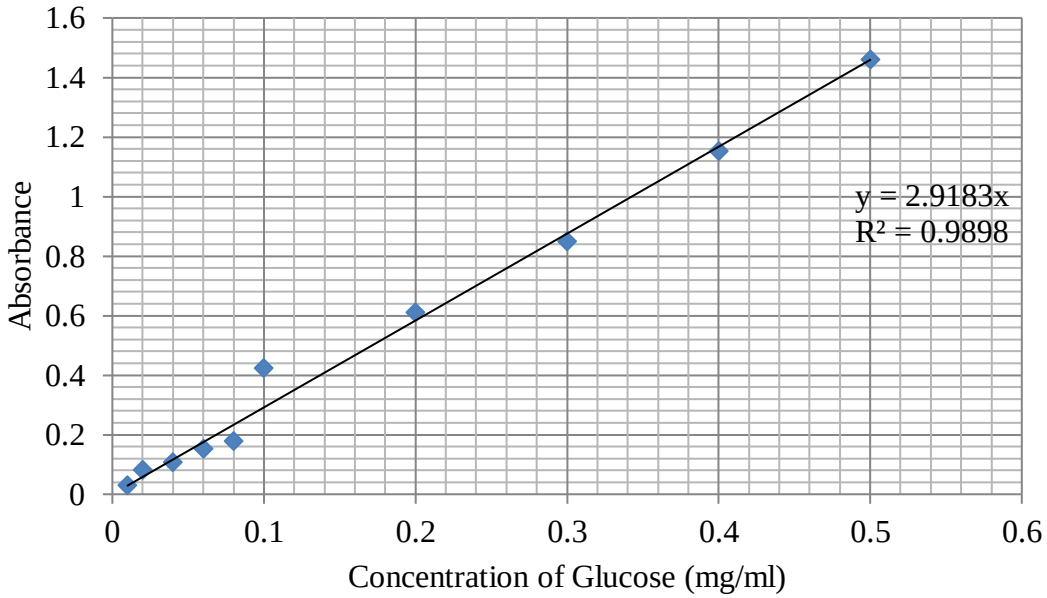
2: Protocol for the Determination of Standard Protein

Reagent	**Blank**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
BSA (mg/ml)	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9	1.0
Distilled Water (ml)	1.0	0.9	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1	0.0
Biuret Reagent (ml)	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Total Volume of Reaction (ml)		5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0

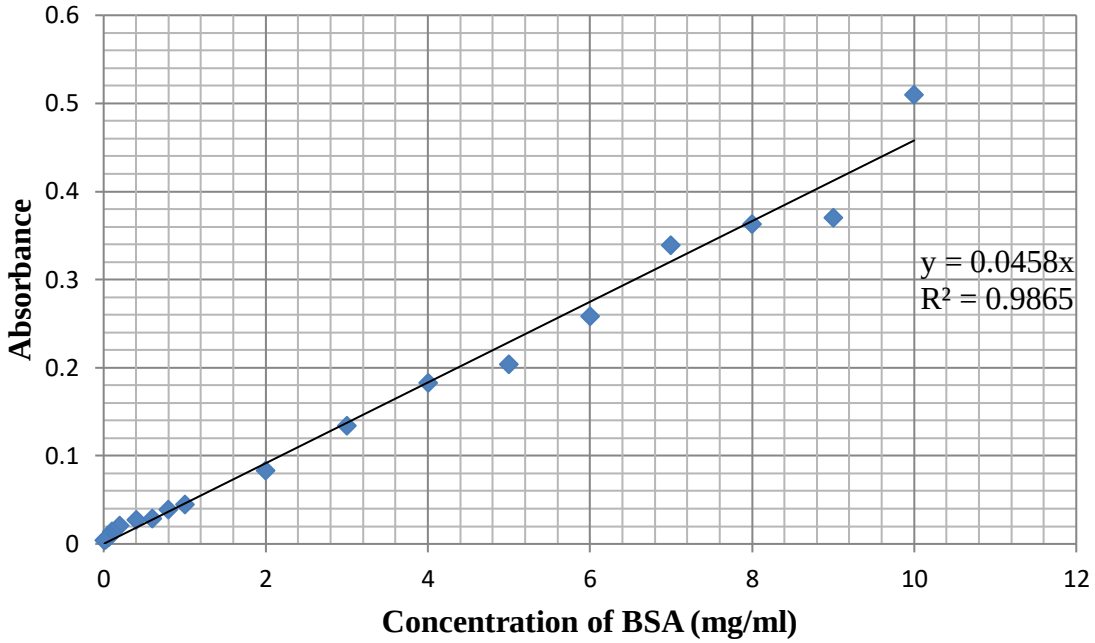
APPENDIX III

STANDARD PLOTS

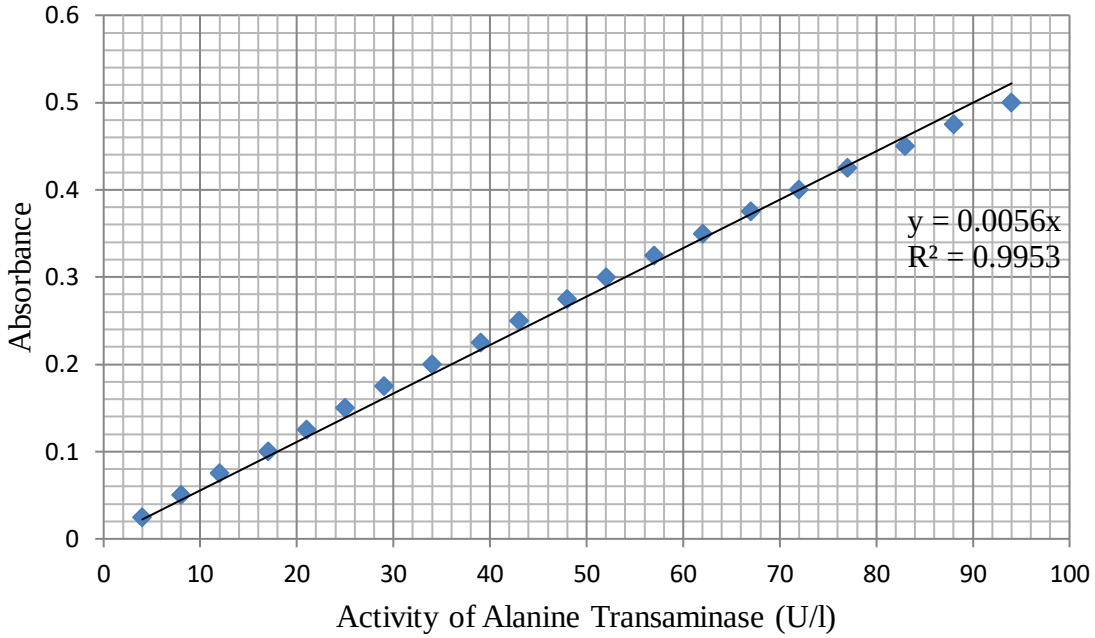
3a: Standard Calibration Curve for Glucose



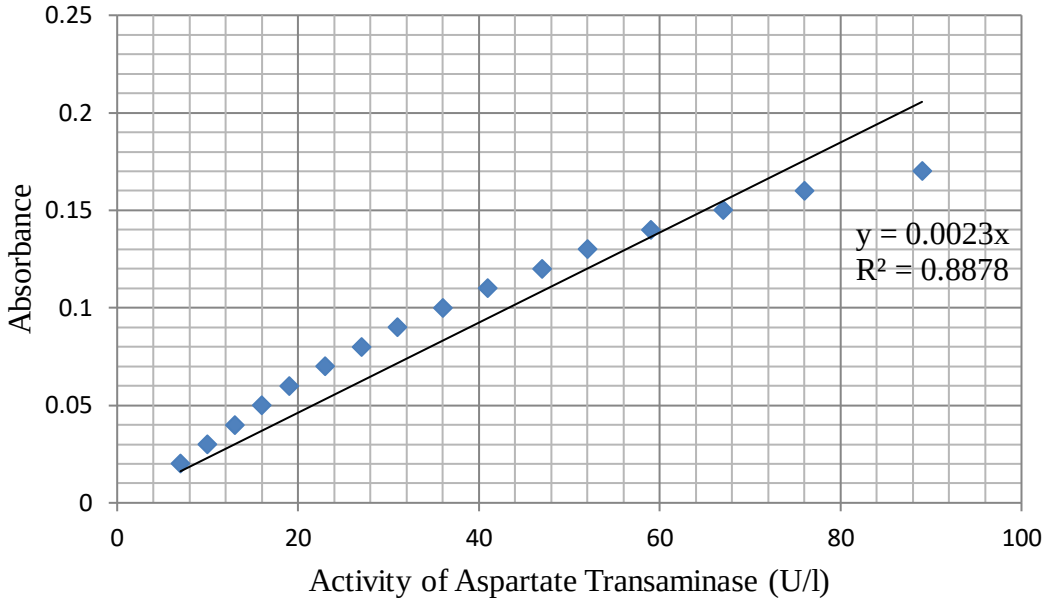
3b: Standard Calibration Curve for the Protein (Bovine Serum Albumin; BSA)



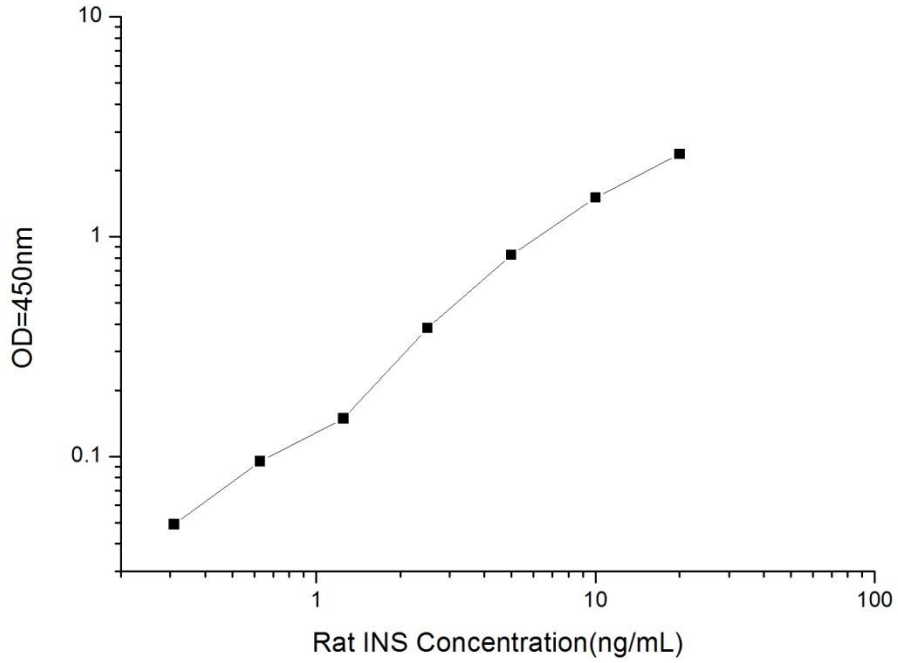
3c: Standard Calibration Curve for Alanine Aminotransferase



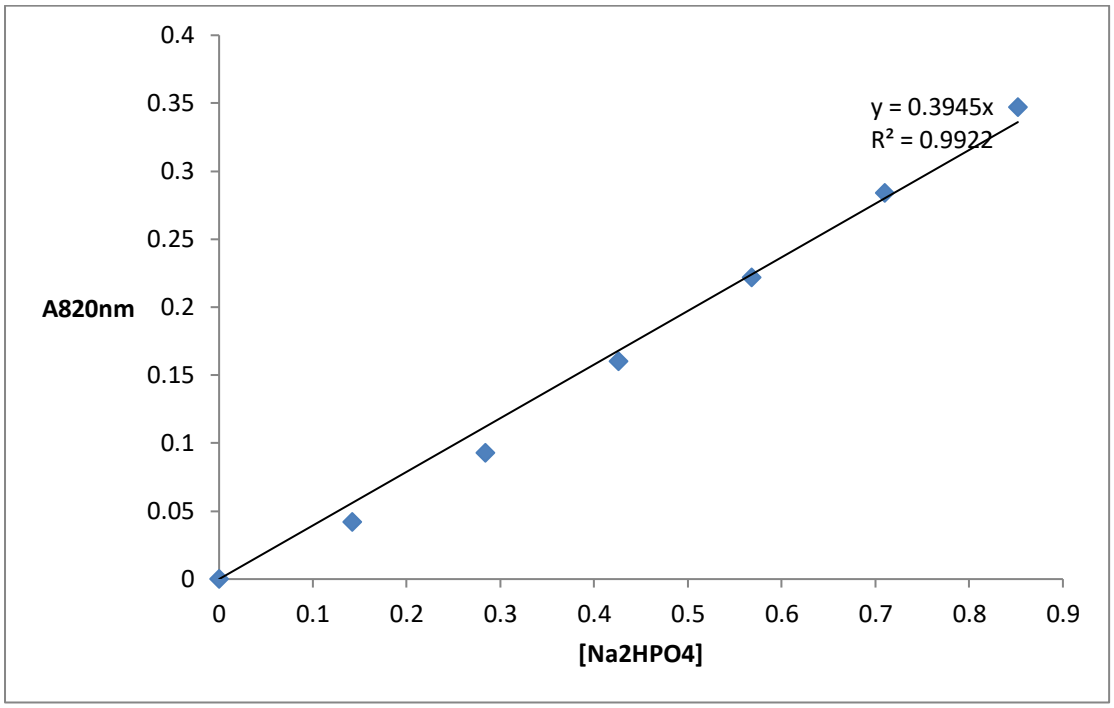
3d: Standard Calibration Curve for Aspartate Aminotransferase



3e: Standard Curve for Rat Insulin



3f: Standard Curve for Phosphate



APPENDIX IV

Ethical Approval

UNIVERSITY OF ILORIN, ILORIN, NIGERIA
UNIVERSITY ETHICAL REVIEW COMMITTEE

Vice-Chancellor: Prof. A.G. Ambali
DVM (ABU), M.V. Sc., Ph.D (Liverpool, UK),
MVCN, MCVSN, MNVMA, FCVSN
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Our Ref: UIL/UERC/06/55EH203

Date: 8TH June, 2017

Protocol Identification Code: UERC/LSC/128
UERC Approval Number: UERC/ASN/2017/907

EVALUATION OF ANTI-DIABETIC ACTIVITY AND TOXICOLOGICAL EFFECTS OF ACQUEOUS EXTRACTS *CHRYSOPHYLLUM ALBIDUM* STEM BARK ON ALBINO RATS

Name of applicant/Principal Investigator: **YUSUF**, Babalola Ola
Address of Applicant: Department of Biochemistry
Faculty of Life Sciences,
University of Ilorin, Ilorin.

Type of Review: Full Committee Review...
Date of Approval: 08/06/2017

Notice of Full Committee Approval

I am pleased to inform you that the research described in the submitted proposal has been reviewed by the University Ethical Review Committee (UERC) and given full Committee approval.

This approval dates from 08/06/2017 to 07/06/2020, and there should be no participant accrual or any activity related to this research to be conducted outside these dates.

You are requested to inform the committee at the commencement of the research to enable it appoints its representative who will ensure compliance with the approved protocol. If there is any delay in starting the research, please inform the UERC so that the dates of approval can be adjusted accordingly.

The UERC requires you to comply with all institutional guidelines and regulations and ensure that all adverse events are reported promptly to the UERC. No charges are allowed in the research without prior approval by the UERC. Please note that the UERC reserves the right to conduct monitoring/oversight visit to your research site without prior notification.

Thank You,

Ismaila Isah
For: University Ethical Review Committee

"....if it's not ethical, it's not scientific, if it's not scientific it's not ethical"