TITLE PAGE

ANTIDIABETIC PROPERTIES OF TOTAL PHENOL CONTENT OF *Zanthoxylum zanthoxyloides* LEAVES ON ALBINO RATS

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A RESEARCH PROJECT PRESENTED TO THE DEPARTMENT OF BIOCHEMISTRY

SCHOOL OF BIOLOGICAL SCIENCES,

SCHOOL OF POSTGRADUATE STUDIES,

FEDERAL UNIVERSITY OF TECHNOLOGY, OWERRI

IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF MASTERS OF SCIENCE (M.Sc.) DEGREE IN BIOCHEMISTRY

JULY, 2019

CERTIFICATION

We hereby certify that this thesis "**Antidiabetic Properties of Total Phenol Content of *Zanthoxylum zanthoxyloides* Leaves on Albino Rats.**" was carried out by EKWEMBA CHRISTIAN O. (20144912568) and accepted in partial fulfillment for the award of Masters (M.Sc.) degree in Pharmacological Biochemistry in the Department of Biochemistry, Federal University of Technology, Owerri.

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DEDICATION

This project work is dedicated to God Almighty.

ACKNOWLEDGEMENTS

A research of this nature cannot be complete without a note of appreciation to those who contributed to its success.

I am grateful to my project supervisors, Prof. N., Nwachukwu and Dr C.O., Ibegbulem, for their assistance and scholastic guidance in this research work. God bless you both.

In a special way, I acknowledge the efforts of my family starting from my parents, Mr and Mrs Fredrick Ekwemba and siblings who in one way or the other contributed to the who and what I am today.

My gratitude also go to my mentors in life, and in the field of Biochemistry, who have impacted into me morally, financially and academically; Prof. C.S. Alisi, Dr J.C., Ifemeje, Mr Francis Ekwemba, Rev. Fr Ambrose Dada, Pastor I.K., Onyeka, and a host of others who time and space would not permit me to list. I am sincerely grateful to you all.

Above all, I am highly grateful to Almighty God whose wisdom, knowledge, understanding and enabling grace have helped me to carry out this project. To Him be all the glory.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

ABSTRACT

In this study, we evaluated the effect of the total phenol extract of *Zanthoxylum zanthoxyloides* leaves on oral glucose tolerance, lipid profile, liver function, oxidative stress parameters, and pancreas histopathology in diabetic rats. Diabetes was induced in rats by administration of alloxan monohydrate in a dose of 130 mg/kg b/w. Total phenol content of *Z. zanthoxyloides* leaves was administered to rats at 100, 200 and 400 mg/kg b/w daily for 15 days. Blood glucose was measured by glucose oxidase method; lipid profile, liver function and oxidative stress parameters were monitored by standard methods and pancreatic histopathology evaluated microscopically. The results showed an increase in blood glucose level of the rats after administration of alloxan monohydrate. Two weeks after treatment with *Z. zanthoxyloides*, a significant ($p<0.05$) reduction in blood glucose was recorded within the treatment groups compared to the untreated control group. Total cholesterol and low density lipoproteins (LDL) were significantly ($p<0.05$) lower in the treated groups, with lowest values recorded in the group treated with the highest concentration of the extract. The result also showed that aspartate aminotransferase (AST), alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities were lowest on treatment with highest concentration of the phenol extract, while glutathione (GSH) and vitamin C levels were increased in the treated groups compared to the diabetic and untreated groups. The result of the pancreatic histopathology further supported the protective effect of *Z. zanthoxyloides* phenol extract towards diabetic damage. From these results, we suggested that total phenolics of *Z. zanthoxyloides* may be effective in controlling blood glucose level in diabetics, and in protecting pancreatic tissues from diabetic damage.

Keywords: Phenol content, lipid profile, liver function, oxidative stress, alloxan.

CHAPTER ONE
INTRODUCTION

1.1 Background of the Study

Diabetes mellitus is a group of metabolic disorders with one common manifestation; hyperglycaemia (World Health Organization [WHO], 1980; World Health Organization [WHO], 1985). It is a major metabolic syndrome characterized by derangement in carbohydrate metabolism associated with defect in insulin secretion or action. Diabetes mellitus is a chronic metabolic disease that is showing an alarming increase in prevalence in developing countries such as Nigeria. Chronic hyperglycaemia causes damage to eyes, kidneys, nerves, heart and blood vessels (Mayfield, 1998). It is caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. It results either from inadequate secretion of hormone insulin, an inadequate response of target cells to insulin, or a combination of these factors. Diabetes is a major risk factor for the development of cardiovascular disease. More than 70% of deaths in diabetic patients are due to vascular disease. One of the greatest factors in the development and progression of the complications of diabetes mellitus is hyperglycaemia (Fiorentino, Prioletta, Zuo & Folli, 2013). This contributes to majority of diabetic complications by altering vascular cellular metabolism, vascular matrix molecules, and circulating lipoproteins (American Diabetes Association, 2010).

Treatment of diabetes involves use of drugs that reduce glucose levels, including insulin and oral antihyperglycaemic drugs. Although there is treatment for diabetes mellitus, most drugs in current use are seriously constrained by both their side effects and cost of treatment. Due to these challenges, populations mainly in Sub-Saharan Africa have resorted to cheaper and readily available alternative sources of treatment, such as use of medicinal plants or traditional medicines (Modak, Dixit, Londhe, Ghaskadbi & Devasagayam, 2007). The World Health Organization (WHO) estimates that 80% of the worlds' populations use traditional medicine. The continued use of traditional medicines is linked to their low cost and a general belief that they have minimal side effects (Somani, Kasture & Singhai, 2006). This disease requires medical diagnosis, treatment and changes in life style. It is projected to become one of the world's main disablers and killers within the next 25 years. The management of diabetes is a global problem, and until now successful treatment is not yet discovered. There are many synthetic medicines developed for patients, but it is the fact that it has never been reported that someone had recovered totally from diabetes (Li, Zheng & Bukuru 2004). The modern oral hypoglycaemic agents produce side effects. Thus, alternative therapy is required.

The need of the hour is therefore to shift towards the different indigenous plant and herbal formulations (Satyanarayana, Katyayani & Latha, 2006).

Recently, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically as antidiabetic and antihyperlipidemic remedies. Despite the presence of known antidiabetic medicine in the pharmaceutical market, diabetes and the related complications continued to be a major medical problem. Antihyperglycaemic effects of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin dependent processes. More than 400 plant species having hypoglycaemic activity have been available in literature, however, searching for new antidiabetic drugs from natural plants is still attractive because they contain substances which demonstrate alternative and safe effects on diabetes mellitus. Most of plants contain glycosides, alkaloids, terpenoids, flavonoids, carotenoids, etc., that are frequently implicated as having antidiabetic effect.

Medicinal plants and their bioactive constituents are used for the treatment of diabetes mellitus throughout the world, especially in countries where access to the conventional treatment of diabetes mellitus is inadequate (Punitha, Vasudevan & Manoharan, 2006). Recently the medicinal values of such plant extracts have been studied by many scientists in the field of diabetic research (Daisy and Eliza, 2007; Noor, Gunasekaran, Manickam & Vijayalakshmi, 2008). Various parts of medicinal plants including the roots, bark and leaves have been used for medicinal purposes, including the treatment of diabetes mellitus, stomachache, toothache, coughs, urinary and venereal diseases, leprous ulcerations, rheumatism, and lumbago, (Olatunji, 1983).

Nigerian *Zanthoxylum* is a common plant found in the rain forest vegetation of southern Nigeria, and is represented by eleven species. A few of these species occur more abundantly in the savannah and dry forest vegetation of south- western Nigeria. The eleven *Zanthoxylum* species demonstrate very close similarities and relationships among themselves, and are identified as trees, erect shrubs or small tree, straggling or scandent shrubs or as a forest liana. Most traditional healers throughout Nigeria have used species of the *Zanthoxylum* for the treatment of a wide range of disorders, including toothache, urinary and venereal diseases, rheumatism and lumbago. Metabolites isolated from *Zanthoxylum* species include alkaloids, aliphatic and aromatic amides, lignins, coumarins, sterols, carbohydrate residues, etc. Some of these metabolites have shown cytotoxic, molluscidal, anticonvulsant, anti-sicking, anesthetic, antibacterial, antihypertensive and anti-inflammatory properties. Crude aqueous

extracts of the root bark of *Zanthoxylum zanthoxyloides* is used in folklore medicine for its anti-inflammatory activity. It is now well established by pharmacological study that the extract has anti-inflammatory activity (Oriowo, 1982). The present study is designed to evaluate the effect of phenolic extracts of *Zanthoxylum zanthoxyloides* leaves on blood glucose level, lipid profile, activities of some plasma liver enzymes and oxidative stress parameters.

1.2 Statement of the Problem

The number of people with diabetes has risen from 108 million in 1980 to 422 million in 2014 (WHO [World Health Organization], 2016). The global prevalence of diabetes among adults over 18 years of age has risen from 4.7% in 1980 to 8.5% in 2014. Diabetes prevalence has been rising more rapidly in middle- and low-income countries. Diabetes is a major cause of blindness, kidney failure, heart attacks, stroke and lower limb amputation. In 2012, an estimated 1.5 million deaths were directly caused by diabetes and another 2.2 million deaths were attributable to high blood glucose. Almost half of all deaths attributable to high blood glucose occur before the age of 70 years (WHO, 2016). WHO projects that diabetes will be the 7th leading cause of death in 2030 (Mathers and Loncar, 2006).

In the past three decades, despite the significant progress made in the treatment of diabetes, the results of treatment in patients is still far from perfect. These treatments have some disadvantages, including drug resistance (reduction of efficiency), side effects, and even toxicity. For example, sulfonylureas lose their effectiveness after 6 years of treatment in 44% of patients. It is also said that the glucose-lowering drugs are not able to control hyperlipidemia (Dey, Attele & Yuan 2002). In addition, the side effects of medicines and their interactions with each other invitro must be considered by medical staff. Today, many treatments that involve the use of medicinal plants are recommended (Kooti *et al.*, 2015). Most plants contain carotenoids, flavonoids, terpenoids, alkaloids, glycosides and can often have anti-diabetic effects (Afrisham, Aberomand, Ghaffari, Siahpoosh & Jamalan, 2015). The anti-hyperglycaemic effects that results from treatment with plants are often due to their ability to improve the performance of pancreatic tissue, which is done by increasing insulin secretions or reducing the intestinal absorption of glucose. The number of people with diabetes today has been growing and causing increasing concerns in medical community and the public. Thus, the main purpose of this research is to search for an effective medicinal plant used for treating diabetes.

1.3 Justification of the Study

Phenolic compounds, for instance phenolic acids and flavonoids, could promote health benefits by reducing the risk of metabolic syndrome and the related complications of type 2 diabetes. However, different groups of phenolic compounds have different biological characteristics, and very little is known about the mechanisms by which they could contribute to the prevention of disease. There is need for further studies.

Many studies have reported the advantages of phenolic compounds, such as anti-aging, anti-inflammatory, antioxidant and antiproliferative activities. In addition to the above reports, there are relevant antioxidant enzymes to counter oxidants (Shukitt-Hale, Lau & Joseph, 2008; Moo-Huchin *et al.,* 2015). Polyphenols, especially flavonoids, phenolic acids and tannins, have the important property of inhibiting α-glucosidase and α-amylase, which are key enzymes responsible for the digestion of dietary carbohydrates to glucose. Dietary plant polyphenols and polyphenol-rich products modulate carbohydrate and lipid metabolism, lower hyperglycaemia, dyslipidemia and insulin resistance, improve β-cell function, stimulate insulin secretion, improve adipose tissue metabolism and alleviate oxidative stress, stress-sensitive signaling pathways and inflammatory processes. Polyphenolic compounds can also prevent the development of long-term diabetes complications, including cardiovascular disease, neuropathy, nephropathy and retinopathy.

Furthermore, phenolic compounds that are found in beverages, vegetables, galenical pears and berries, may facilitate fitness by decreasing the risk of metabolic syndrome and relevant complications of type 2 diabetes (Dembinska-Kiec, Mykkänen, Kiec-Wilk & Mykkänen, 2008). Since different crude extracts of the *Zanthoxylum zanthoxyloides* plant have proved to be of numerous traditional, nutritional and medicinal values including the treatment and control of diabetes, it becomes imperative that we undertake a study on the possible role of the phenolic contentsin diabetes management.

1.4 Aim of Study

The aim of this study was to evaluate the antidiabetic properties of total phenol contents of *Z. zanthoxyloides* leaves in albino rats.

1.5 Specific Objectives of the Study

The specific objectives of this study were to:

i. determine serum glucose levels of diabetic rats treated with *Zanthoxylum zanthoxyloides* phenolic leaves fraction;

ii. determine lipid profile of diabetic rats treated with *Zanthoxylum zanthoxyloides* phenolic leaves fraction of diabetic rats treated with *Zanthoxylum zanthoxyloides* phenolic leaves fraction;

iii. determine liver function enzymes of diabetic rats treated with *Zanthoxylum zanthoxyloides* phenolic leaves fraction;

iv. determine oxidative stress parameters of diabetic rats treated with *Zanthoxylum zanthoxyloides* phenolic leaves fraction.

CHAPTER TWO
LITERATURE REVIEW

2.1 Diabetes Mellitus

Diabetes mellitus (**DM**), commonly referred to as **diabetes**, is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period (World Health Organisation [WHO], 2014). Symptoms of high blood sugar include frequent urination, increased thirst, and increased hunger. If left untreated, diabetes can cause many complications (World Health Organisation [WHO], 2013). Acute complications can include diabetic ketoacidosis, nonketotic hyperosmolar coma, or death (Kitabchi, Umpierrez, Miles & Fisher, 2009). Serious long-term complications include heart disease, stroke, chronic kidney failure, foot ulcers, and damage to the eyes (WHO, 2013).

Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced (David, 2011). The three main types of diabetes mellitus are Type 1 DM, Type 2 DM and Gestational diabetes.

Prevention and treatment involve maintaining a healthy diet, regular physical exercise, a normal body weight, and avoiding use of tobacco. Control of blood pressure and maintaining proper foot care are important for people with the disease. Type 1 DM must be managed with insulin injections (WHO, 2013). Type 2 DM may be treated with medications with or without insulin (WHO, 2013). Insulin and some oral medications can cause low blood sugar (Richard and James, 2010). Weight loss surgery in those with obesity is sometimes an effective measure in those with type 2 DM (Picot *et al.,* 2009). Gestational diabetes usually resolves after the birth of the baby (Cash, 2014).

As at 2015, an estimated 415 million people had diabetes worldwide (International Diabetes Federation [IDF], 2016), with type 2 DM making up about 90% of the cases (Shi and Hu, 2014). This represents 8.3% of the adult population (Vos *et al.,* 2012), with equal rates in both women and men (Vos *et al.,* 2012). As at 2014, trends suggested that the rate would continue to rise (IDF, 2016). Diabetes at least doubles a person's risk of early death (WHO, 2013). From 2012 to 2015, approximately 1.5 to 5.0 million deaths each year resulted from diabetes (WHO, 2013; IDF, 2016). The global economic cost of diabetes in 2014 was estimated to be US$612 billion (IDF, 2013). In the United States, diabetes cost $245 billion in 2012 (International Diabetes Federation [IDF], 2013).

2.1.1 Types of Diabetes Mellitus

Diabetes mellitus is classified into four broad categories: type 1, type 2, gestational diabetes, and "other specific types" (David, 2011). The "other specific types" are a collection of a few

dozen individual causes (David, 2011). Diabetes is a more variable disease than once thought and people may have combinations of forms (Tuomi *et al.*, 2014). The term "diabetes", without qualification, usually refers to diabetes mellitus.

Type 1

Type 1 diabetes mellitus is characterized by loss of the insulin-producing beta cells of the islets of Langerhans in the pancreas, leading to insulin deficiency. This type can be further classified as immune-mediated or idiopathic. The majority of type 1 diabetes is of the immune-mediated nature, in which a T-cell-mediated autoimmune attack leads to the loss of beta cells and thus insulin (Rother, 2007). It causes approximately 10% of diabetes mellitus cases in North America and Europe. Most affected people are otherwise healthy and of a healthy weight when onset occurs. Sensitivity and responsiveness to insulin are usually normal, especially in the early stages. Type 1 diabetes can affect children or adults, but was traditionally termed "juvenile diabetes" because a majority of these diabetes cases were in children.

"Brittle" diabetes, also known as unstable diabetes or labile diabetes is a term that was traditionally used to describe the dramatic and recurrent swings in glucose levels, often occurring for no apparent reason in insulin-dependent diabetes. This term, however, has no biologic basis and should not be used. Still, type 1 diabetes can be accompanied by irregular and unpredictable high blood sugar levels, frequently with ketosis, and sometimes with serious low blood sugar levels. Other complications include an impaired counterregulatory response to low blood sugar, infection, gastroparesis (which leads to erratic absorption of dietary carbohydrates), and endocrinopathies (e.g., Addison's disease). These phenomena are believed to occur no more frequently than in 1% to 2% of persons with type 1 diabetes (Dorner, Pinget & Brogard, 1977).

Type 1 diabetes is partly inherited, with multiple genes, including certain HLA genotypes, known to influence the risk of diabetes. The increase of incidence of type 1 diabetes reflects the modern lifestyle (Phillips, Couper, Penno & Harrison, 2016). In genetically susceptible people, the onset of diabetes can be triggered by one or more environmental factors (Petzold *et al.*, 2015), such as a viral infection or diet. Several viruses have been implicated, but to date there is no stringent evidence to support this hypothesis in humans (Butalia, Kaplan, Khokhar & Rabi, 2016). Among dietary factors, data suggest that gliadin (a protein present in gluten) may play a role in the development of type 1 diabetes, but the mechanism is not fully understood (Serena, Camhi, Sturgeon, Yan & Fasano, 2015; Visser, Rozing, Sapone, Lammers & Fasano, 2009).

Type 2

Type 2 DM is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion (David, 2011). The defective responsiveness of body tissues to insulin is believed to involve the insulin receptor. However, the specific defects are not known. Diabetes mellitus cases due to a known defect are classified separately. Type 2 DM is the most common type of diabetes mellitus.

In the early stage of type 2, the predominant abnormality is reduced insulin sensitivity. At this stage, high blood sugar can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce the liver's glucose production.

Type 2 DM is due primarily to lifestyle factors and genetics (Risérus, Willett & Hu, 2009). A number of lifestyle factors are known to be important to the development of type 2 DM, including obesity (defined by a body mass index of greater than 30), lack of physical activity, poor diet, stress, and urbanization. Excess body fat is associated with 30% of cases in those of Chinese and Japanese descent, 60–80% of cases in those of European and African descent, and 100% of Pima Indians and Pacific Islanders (David, 2011). Even those who are not obesed often have a high waist–hip ratio (David, 2011).

Dietary factors also influence the risk of developing type 2 DM. Consumption of sugar-sweetened drinks in excess is associated with an increased risk (Malik, Popkin, Bray, Després & Hu, 2010a; Malik, Popkin, Bray, Després & Hu, 2010b). The type of fats in the diet is also important, with saturated fats and trans fatty acids increasing the risk and polyunsaturated and monounsaturated fat decreasing the risk (Risérus *et al.*, 2009). Eating lots of white rice also may increase the risk of diabetes (Hu, Pan, Malik & Sun, 2012). A lack of exercise is believed to cause 7% of cases (Lee *et al.*, 2012).

Gestational Diabetes

Gestational diabetes mellitus (GDM) resembles type 2 DM in several respects, involving a combination of relatively inadequate insulin secretion and responsiveness. It occurs in about 2–10% of all pregnancies and may improve or disappear after delivery (U.S. Department of Health and Human Services [USDHHS], 2014). However, after pregnancy approximately 5–10% of women with gestational diabetes are found to have diabetes mellitus, most commonly type 2 (USDHHS, 2014). Gestational diabetes is fully treatable, but requires careful medical supervision throughout the pregnancy. Management may include dietary changes, blood glucose monitoring, and in some cases, insulin may be required.

Though it may be transient, untreated gestational diabetes can damage the health of the fetus or mother. Risks to the baby include macrosomia (high birth weight), congenital heart and

central nervous system abnormalities, and skeletal muscle malformations. Increased levels of insulin in a fetus's blood may inhibit fetal surfactant production and cause respiratory distress syndrome. A high blood bilirubin level may result from red blood cell destruction. In severe cases, perinatal death may occur, most commonly as a result of poor placental perfusion due to vascular impairment. Labor induction may be indicated with decreased placental function. A Caesarean section may be performed if there is marked fetal distress or an increased risk of injury associated with macrosomia, such as shoulder dystocia.

Other Types

Prediabetes indicate conditions that occurs when blood glucose levels are higher than normal but not high enough for a diagnosis of type 2 DM. Many people destined to develop type 2 DM spend many years in a state of prediabetes.

Latent autoimmune diabetes of adults (LADA) is a condition in which type 1 DM develops in adults. Adults with LADA are frequently initially misdiagnosed as having type 2 DM, based on age rather than etiology.

Some cases of diabetes are caused by the body's tissue receptors not responding to insulin (even when insulin levels are normal, which is what separates it from type 2 diabetes); this form is very uncommon. Genetic mutations (autosomal or mitochondrial) can lead to defects in beta cell function. Abnormal insulin action may also have been genetically determined in some cases. Any disease that causes extensive damage to the pancreas may lead to diabetes (for example, chronic pancreatitis and cystic fibrosis). Diseases associated with excessive secretion of insulin-antagonistic hormones can cause diabetes (which is typically resolved once the hormone excess is removed). Many drugs impair insulin secretion and some toxins damage pancreatic beta cells. The International Classification of Diseases 10th Revision (ICD-10) (1992) diagnostic entity, *malnutrition-related diabetes mellitus* (MRDM or MMDM, ICD-10 code E12), was deprecated by the World Health Organization when the current taxonomy was introduced in 1999 (World Health Organization [WHO], 1999).

Other forms of diabetes mellitus include congenital diabetes, which is due to genetic defects of insulin secretion, cystic fibrosis-related diabetes, steroid diabetes induced by high doses of glucocorticoids, and several forms of monogenic diabetes.

"Type 3 diabetes" has been suggested as a term for Alzheimer's disease as the underlying processes may involve insulin resistance by the brain (De la Monte, 2014).

2.1.2 Signs and Symptoms of Diabetes Mellitus

The classic symptoms of untreated diabetes are weight loss, polyuria (increased urination), polydipsia (increased thirst), and polyphagia (increased hunger) (Cooke and Plotnick, 2008).

Symptoms may develop rapidly (weeks or months) in type 1 DM, while they usually develop much more slowly and may be subtle or absent in type 2 DM.

Several other signs and symptoms can mark the onset of diabetes although they are not specific to the disease. In addition to the known ones above, they include blurry vision, headache, fatigue, slow healing of cuts, and itchy skin. Prolonged high blood glucose can cause glucose absorption in the lens of the eye, which leads to changes in its shape, resulting in vision changes. A number of skin rashes that can occur in diabetes are collectively known as diabetic dermadromes.

Diabetic Emergencies

Low blood sugar is common in persons with type 1 and type 2 DM. Most cases are mild and are not considered medical emergencies. Effects can range from feelings of unease, sweating, trembling, and increased appetite in mild cases to more serious issues such as confusion, changes in behavior such as aggressiveness, seizures, unconsciousness, and (rarely) permanent brain damage or death in severe cases (Kenny, 2014; Verrotti, Scaparrotta, Olivieri & Chiarelli, 2012). Moderate hypoglycemia may easily be mistaken for drunkenness (Hsieh, 2016); rapid breathing and sweating, cold, pale skin are characteristic of hypoglycemia but not definitive. Mild to moderate cases are self-treated by eating or drinking something high in sugar. Severe cases can lead to unconsciousness and must be treated with intravenous glucose or injections with glucagon.

People (usually with type 1 DM) may also experience episodes of diabetic ketoacidosis, a metabolic disturbance characterized by nausea, vomiting and abdominal pain, the smell of acetone on the breath, deep breathing known as Kussmaul breathing, and in severe cases a decreased level of consciousness (Kitabchi *et al.,* 2009).

A rare but equally severe possibility is hyperosmolar nonketotic state, which is more common in type 2 DM and is mainly the result of dehydration (Kitabchi *et al.,* 2009).

Complications

All forms of diabetes increase the risk of long-term complications. These typically develop after many years (10–20), but may be the first symptom in those who have otherwise not received a diagnosis before that time.

The major long-term complications relate to damage to blood vessels. Diabetes doubles the risk of cardiovascular disease (Sarwar *et al.,* 2010) and about 75% of deaths in diabetics are due to coronary artery disease (O'Gara *et al.,* 2013). Other "macrovascular" diseases are stroke, and peripheral vascular disease.

The primary complications of diabetes due to damage in small blood vessels include damage to the eyes, kidneys, and nerves (WHO, 2014). Damage to the eyes, known as diabetic retinopathy, is caused by damage to the blood vessels in the retina of the eye, and can result in gradual vision loss and blindness (WHO, 2014). Damage to the kidneys, known as diabetic nephropathy, can lead to tissue scarring, urine protein loss, and eventually chronic kidney disease, sometimes requiring dialysis or kidney transplant (WHO, 2014). Damage to the nerves of the body, known as diabetic neuropathy, is the most common complication of diabetes (WHO, 2014). The symptoms can include numbness, tingling, pain, and altered pain sensation, which can lead to damage to the skin. Diabetes-related foot problems (such as diabetic foot ulcers) may occur, and can be difficult to treat, occasionally requiring amputation. Additionally, proximal diabetic neuropathy causes painful muscle wasting and weakness.

There is a link between cognitive deficit and diabetes. Compared to those without diabetes, those with the disease have a 1.2 to 1.5-fold greater rate of decline in cognitive function (Cukierman, 2005).

2.1.3 Pathophysiology of Diabetes Mellitus

Insulin is the principal hormone that regulates the uptake of glucose from the blood into most cells of the body, especially liver, muscle, and adipose tissue. Therefore, deficiency of insulin or the insensitivity of its receptors plays a central role in all forms of diabetes mellitus (American Diabetes Association, 2014).

The body obtains glucose from three main places: the intestinal absorption of food, the breakdown of glycogen, the storage form of glucose found in the liver, and gluconeogenesis, the generation of glucose from non-carbohydrate substrates in the body (David, 2011). Insulin plays a critical role in balancing glucose levels in the body. Insulin can inhibit the breakdown of glycogen or the process of gluconeogenesis, it can stimulate the transport of glucose into fat and muscle cells, and it can stimulate the storage of glucose in the form of glycogen (David, 2011).

Insulin is released into the blood by beta cells (β-cells), found in the islets of Langerhans in the pancreas, in response to rising levels of blood glucose, typically after eating. Insulin is used by about two-thirds of the body's cells to absorb glucose from the blood for use as fuel, for conversion to other needed molecules, or for storage. Lower glucose levels result in decreased insulin release from the beta cells and in the breakdown of glycogen to glucose. This process is mainly controlled by the hormone glucagon, which acts in the opposite manner to insulin (Kim, 2012).

If the amount of insulin available is insufficient, if cells respond poorly to the effects of insulin (insulin insensitivity or insulin resistance), or if the insulin itself is defective, then glucose will not be absorbed properly by the body cells that require it, and it will not be stored appropriately in the liver and muscles. The net effect is persistently high levels of blood glucose, poor protein synthesis, and other metabolic derangements, such as acidosis (David, 2011).

When the glucose concentration in the blood remains high over time, the kidneys will reach a threshold of reabsorption, and glucose will be excreted in the urine (glycosuria) (Robert, 2012). This increases the osmotic pressure of the urine and inhibits reabsorption of water by the kidney, resulting in increased urine production (polyuria) and increased fluid loss (David, 2011).

2.1.4 Diagnosis of Diabetes Mellitus

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

- Fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl)
- Plasma glucose ≥ 11.1 mmol/l (200 mg/dl) two hours after a 75 g oral glucose load as in a glucose tolerance test
- Symptoms of high blood sugar and casual plasma glucose ≥ 11.1 mmol/l (200 mg/dl)

Glycated hemoglobin (HbA_{1C}) ≥ 48 mmol/mol (≥ 6.5 DCCT %) (American Diabetes Association, 2010).

A positive result, in the absence of unequivocal high blood sugar, should be confirmed by a repeat of any of the above methods on a different day. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test (Saydah *et al.,* 2001). According to the current definition, two fasting glucose measurements above 126 mg/dl (7.0 mmol/l) are considered diagnostic for diabetes mellitus.

For the World Health Organization, people with fasting glucose levels from 6.1 to 6.9 mmol/l (110 to 125 mg/dl) are considered to have impaired fasting glucose (World Health Organization [WHO], 2006). People with plasma glucose at or above 7.8 mmol/l (140 mg/dl), but not over 11.1 mmol/l (200 mg/dl), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two prediabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus, as well as cardiovascular disease (Santaguida *et al.,* 2008). The American Diabetes Association since 2003 uses a

slightly different range for impaired fasting glucose of 5.6 to 6.9 mmol/l (100 to 125 mg/dl) (Bartoli, Fra & Carnevale Schianca, 2011).

Glycated hemoglobin is better than fasting glucose for determining risks of cardiovascular disease and death from any cause (Selvin *et al.*, 2010).

The rare disease diabetes insipidus has similar symptoms to diabetes mellitus, but without disturbances in the sugar metabolism (*insipidus* means "without taste" in Latin) and does not involve the same disease mechanisms. Diabetes is a part of the wider condition known as metabolic syndrome.

2.1.5 Prevention of Diabetes Mellitus

There is no known preventive measure for type 1 diabetes (WHO, 2013). Type 2 diabetes — which accounts for 85-90% of all cases — can often be prevented or delayed by maintaining a normal body weight, engaging in physical exercise, and consuming a healthful diet (WHO, 2013). Higher levels of physical activity reduce the risk of diabetes by 28% (Kyu *et al.*, 2016). Dietary changes known to be effective in helping to prevent diabetes include maintaining a diet rich in whole grains and fiber, and choosing good fats, such as the polyunsaturated fats found in nuts, vegetable oils, and fish. Limiting sugary beverages and eating less red meat and other sources of saturated fat can also help prevent diabetes. Tobacco smoking is also associated with an increased risk of diabetes and its complications, so smoking cessation can be an important preventive measure as well (Willi, Bodenmann, Ghali, Faris & Cornuz, 2007).

The relationship between type 2 diabetes and the main modifiable risk factors (excess weight, unhealthy diet, physical inactivity and tobacco use) is similar in all regions of the world. There is growing evidence that the underlying determinants of diabetes are a reflection of the major forces driving social, economic and cultural change: globalization, urbanization, population ageing, and the general health policy environment (WHO, 2016).

2.1.6 Management of Diabetes Mellitus

Diabetes mellitus is a chronic disease, for which there is no known cure except in very specific situations. Management concentrates on keeping blood sugar levels as close to normal, without causing low blood sugar. This can usually be accomplished with a healthy diet, exercise, weight loss, and use of appropriate medications (insulin in the case of type 1 diabetes; oral medications, as well as possibly insulin, in type 2 diabetes).

Learning about the disease and actively participating in the treatment is important, since complications are far less common and less severe in people who have well-managed blood sugar levels (Nathan *et al.*, 2005; Complications Trial Research Group, 1995). The goal of

treatment is to achieve an HbA_{1C} level of 6.5%, but should not be lower than that, and may be set higher (National Institute for Health and Clinical Excellence, 2008). Attention is also paid to other health problems that may accelerate the negative effects of diabetes. These include smoking, elevated cholesterol levels, obesity, high blood pressure, and lack of regular exercise (National Institute for Health and Clinical Excellence, 2008). Specialized footwear is widely used to reduce the risk of ulceration, or re-ulceration, in at-risk diabetic feet. Evidence for the efficacy of this remains equivocal, however (Cavanagh, 2004).

Lifestyle

People with diabetes can benefit from education about the disease and treatment, good nutrition to achieve a normal body weight, and exercise, with the goal of keeping both short-term and long-term blood glucose levels within acceptable bounds. In addition, given the associated higher risks of cardiovascular disease, lifestyle modifications are recommended to control blood pressure (Adler *et al.*, 2004).

Medications

Medications used to treat diabetes do so by lowering blood sugar levels. There are a number of different classes of anti-diabetic medications. Some are available for oral route, such as metformin, while others are only available by injection such as GLP-1 agonists. Type 1 diabetes can only be treated with insulin, typically with a combination of regular and Neutral Protamine Hagedorn (NPH) insulin, or synthetic insulin analogs.

Metformin is generally recommended as a first line treatment for type 2 diabetes, as there is good evidence that it decreases mortality (Ripsin, Kang & Urban, 2009). It works by decreasing the liver's production of glucose (Krentz and Bailey, 2005). Several other groups of drugs, mostly given by oral route, may also decrease blood sugar in type II DM. These include agents that increase insulin release, agents that decrease absorption of sugar from the intestines, and agents that make the body more sensitive to insulin (Krentz and Bailey, 2005). When insulin is used in type 2 diabetes, a long-acting formulation is usually added initially, while continuing oral medications (Ripsin *et al.*, 2009). Doses of insulin are then increased to achieve desired effect (Ripsin *et al.*, 2009).

Since cardiovascular disease is a serious complication associated with diabetes, some have recommended blood pressure levels below 130/80 mmHg (Nelson, 2010). However, evidence supports less than or equal to somewhere between 140/90 mmHg to 160/100 mmHg; the only additional benefit found for blood pressure targets beneath this range was an isolated decrease in stroke risk, and this was accompanied by an increased risk of other serious adverse events (Arguedas, Perez & Wright, 2009; Arguedas, Leiva & Wright, 2013). A 2016

review found potential harm in treating diabetic patients with blood pressure lower than 140 mmHg (Brunström and Carlberg, 2016). Among medications that lower blood pressure, angiotensin converting enzyme inhibitors (ACEIs) improve outcomes in those with DM while the similar medications angiotensin receptor blockers (ARBs) do not (Cheng *et al.*, 2014). Aspirin is also recommended for people with cardiovascular problems, however routine use of aspirin has not been found to improve outcomes in uncomplicated diabetes (Pignone *et al.*, 2010).

Surgery

A pancreas transplant is occasionally considered for people with type 1 diabetes who have severe complications of the disease, including end stage kidney disease requiring kidney transplantation (American Diabetes Association, 2014).

Weight loss surgery in those with obesity and type two diabetes is often an effective measure (Picot *et al.*, 2009). Many are able to maintain normal blood sugar levels with little or no medications following surgery (Frachetti and Goldfine, 2009) and long-term mortality is decreased (Schulman, del Genio, Sinha & Rubino, 2009). There however is some short-term mortality risk of less than 1% from the surgery (Colucci, 2011). The body mass index cutoffs for when surgery is appropriate are not yet clear (Schulman *et al.*, 2009). It is recommended that this option be considered in those who are unable to get both their weight and blood sugar under control (Dixon, le Roux, Rubino & Zimmet, 2012).

Support

In countries using a general practitioner system, such as the United Kingdom, care may take place mainly outside hospitals, with hospital-based specialist care used only in case of complications, difficult blood sugar control, or research projects. In other circumstances, general practitioners and specialists share care in a team approach. Home telehealth support can be an effective management technique (Polisena *et al.*, 2009).

2.2 Liver Enzymes and Diabetes Mellitus

The liver helps maintain normal blood glucose concentration in the fasting and postprandial states. Loss of insulin effect on the liver leads to glycogenolysis and an increase in hepatic glucose production.

Abnormalities of triglyceride storage and lipolysis in insulin-sensitive tissues such as the liver are an early manifestation of conditions characterized by insulin resistance and are detectable earlier than fasting hyperglycaemia. The precise genetic, environmental, and metabolic

factors and sequence of events that lead to the underlying insulin resistance, however, is not fully understood.

Liver function tests (LFTs) are commonly used in clinical practice to screen for liver disease, monitor the progression of known disease, and monitor the effects of potentially hepatotoxic drugs.

The most common LFTs include the serum aminotransferases, alkaline phosphatase, bilirubin and albumin. Aminotransferases, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), measure the concentration of intracellular hepatic enzymes that have leaked into the circulation and serve as a marker of hepatocyte injury. Alkaline phosphatase (AP) and bilirubin act as markers of biliary function and cholestasis. Albumin reflects liver synthetic function.

The aminotransferases AST and ALT are normally < 30–40 units/l. Elevations of aminotransferases greater than eight times the upper limit of normal reflect either acute viral hepatitis, ischemic hepatitis, or drug- or toxin-induced liver injury. Much more common than patients with acute hepatitis, however, are patients with chronic mild elevation of aminotransferases, or AST and ALT < 250 units/l for > 6 months.

Chronic mild elevations of transaminases are frequently found in type 2 diabetic patients.

Salmela, Sotaniemi, Niemi & Maentausta (1984) studied the prevalence of abnormal LFTs and their relationship to clinical findings in 175 unselected diabetic outpatients in Finland. One hundred and eighteen patients were classified as having type 2 diabetes and 57 as having type 1 diabetes. Of those with type 2 diabetes, 33 patients used insulin in addition to diet and oral hypoglycaemic drugs including sulfonylurea and metformin. None of the patients had known chronic liver disease, and none had clinically significant diabetic nephropathy. Hemoglobin A1c (A1C) averaged 11.2 ± 2.4%.

Liver Function Tests measured included albumin, total bilirubin, AST, ALT, AP, GGT, and serum concentrations of cholic acid and chenodeoxycholic acid. Fifty-seven percent of the 175 diabetic outpatients (100 subjects) had at least one abnormal LFT; 27% (48 subjects) had at least two abnormal tests. The type 2 diabetic patients more frequently had elevated ALT (22.9 vs. 5.3%) and GGT (23.7 vs. 10.5%) levels than those with type 1 diabetes. On the other hand, patients with type 1 diabetes more frequently had elevated bilirubin levels (21.1 vs. 10.2%). However, increases in LFTs were rarely more than twice the upper limit of normal.

Multivariate analysis showed BMI > 25 kg/m2 and poor diabetic control (fasting blood glucose > 216 mg/dl) were the most significant clinical variables associated with elevated

ALT and GGT. Elevated ALT was also associated with onset of diabetes within the past 4 years, mature onset of diabetes (35–51 years), and use of diet or sulfonylurea.

To investigate the reliability of LFTs in assessing histological changes, Salmela *et al* (1984) looked at 72 consecutive diabetic in patients with hepatomegaly or abnormal LFTs who were awaiting liver biopsy. Sixty-eight of the patients had type 2 diabetes; four had type 1 diabetes.

All of the patients had hepatomegaly or abnormal LFTs. They had normal blood counts, serum electrolytes, and renal function. None had decompensated heart failure. Only 5 gave a history of social drinking; the other 67 patients were classified as abstainers.

Of the 72 patients who underwent liver biopsy, all 4 with type 1 diabetes had normal liver histology, but only 5 of the 68 with type 2 diabetes had normal liver histology. The most commonly elevated LFT in the nine patients with normal histology included bilirubin and AP. ALT was less frequently elevated, and GGT was not elevated at all. Of the 63 patients with abnormal liver histology, 48 had fatty liver or steatosis with nonspecific inflammatory changes, whereas 14 had evidence of fibrosis. Gamma-glutamyl transferase (GGT) and aspartate aminotransferase (ALT) were most commonly elevated. As histology worsened (steatosis to inflammation to fibrosis), there was no significant difference in mean values of ALT and GGT. Therefore, although abnormal LFT results are common in diabetes, especially in overweight type 2 diabetic patients, they are not reliable in predicting histological changes in the liver.

Ohlson *et al* (1988) found elevated ALT in nondiabetic Swedish men to be a risk factor for type 2 diabetes, independent of obesity, body fat distribution, plasma glucose, lipid, AST, bilirubin concentrations, and family history of diabetes.

With similar results, Vozaroza *et al* (2002) followed 451 nondiabetic Pima Indians for an average of 6.9 years to determine whether hepatic enzyme elevations could be linked to the development of type 2 diabetes. After adjustment for age, sex, body fat, whole body insulin sensitivity, and acute insulin response, only elevated ALT at baseline was associated with an increase in hepatic glucose output. Prospectively, increasing ALT concentrations were associated with a decline in hepatic insulin sensitivity and risk of type 2 diabetes. The authors concluded that higher ALT is a risk factor for type 2 diabetes and indicates a potential role of increased hepatic gluconeogenesis and/or inflammation in the pathogenesis of type 2 diabetes.

2.3 Pancreas and Diabetes Mellitus

The pancreas is a long, soft organ that lies transversely along the posterior abdominal wall, posterior to the stomach, and extends from the region of the duodenum to the spleen. This gland has two major tissue types:

i. An exocrine portion (acini) that secretes digestive enzymes that are carried through a duct to the duodenum
ii. An endocrine portion (islets of Langerhans) which consists of the pancreatic islets that secrete glucagons and insulin

Endocrine tissue contains alpha, beta, and delta cells. Alpha cells in the pancreatic islets secrete the hormone glucagons in response to a low concentration of glucose in the blood. Beta cells secrete the hormone insulin in response to a high concentration of glucose in the blood. Delta cells secrete the hormone somatostatin, which inhibits insulin and glucagon secretion.

Diabetes is the result of a deficiency of insulin, which is found in the endocrine tissue of the pancreas. In people with type 2 diabetes, the pancreas produces insulin but the body is not able to use it. Muscle, fat, and liver cells do not respond to insulin properly. This is a condition known as insulin resistance. People with insulin resistance need more insulin to help glucose enter the cells. The pancreas tries to keep up with the increased demand for insulin, but eventually fails to produce enough to do so. This causes excess glucose to build up in the bloodstream. Over time, the effects of repeated high blood glucose levels will damage beta cells, further reducing the ability of the pancreas to produce insulin.

2.4 Oxidative Stress Parameters and Diabetes

It is believed that oxidative stress plays important role in the development of vascular complications in diabetes particularly type 2 diabetes (Pham-Huy, He & Pham-Huy, 2008). Reactive Oxygen Species (ROS) level elevation in diabetes may be due to increase in destruction or/and decrease in the production by catalase (CAT—enzymatic/non-enzymatic), superoxide dismutase (SOD) and glutathione peroxidase (GSH–Px) antioxidants. The variation in the levels of these enzymes makes the tissues susceptible to oxidative stress leading to the development of diabetic complications (Lipinski, 2001). According to epidemiological studies, diabetic mortalities can be explained notably by an increase in vascular diseases other than hyperglycaemia (Pham-Huy *et al.*, 2008).

2.4.1 Pathophysiology of Oxidative Stress in Diabetes

Nowadays, evidences have been reported that support the role of oxidative stress in the pathogenesis of both type 1 and type 2 diabetes. Free radical formation in diabetes by non-enzymatic glycation of proteins, glucose oxidation and increased lipid peroxidation leads to damage of enzymes, cellular machinery and also increased insulin resistance due to oxidative stress (Maritim, Sanders & Watkins, 2003). According to latest research, lipid is not only but also the apolipoprotein component of LDL that forms insoluble aggregates oxidatively due to hydroxyl radical-induced cross-linkage between apo-B monomers that is responsible for oxidative damage in diabetic complications (Pham-Huy *et al.,* 2008). In diabetes mellitus, main sources of oxidative stress are mitochondria. During oxidative metabolism in mitochondria, a component of the utilized oxygen is reduced to water, and the remaining oxygen is transformed to oxygen free radical ($O_{\bullet}$) which is an important ROS that is converted to other RS such as $ONOO^{-}$, OH and H_2O_2 (Moussa, 2008). Insulin signaling is modulated by ROS/RNS by two ways. On one side, in response to insulin, the ROS/RNS are produced to exert its full physiological function and on the other side, the ROS and RNS have got negative regulation on insulin signaling, interpreting them to develop insulin resistance which is a risk factor for diabetes type 2 (Erejuwa, 2012).

2.4.2 Oxidative Stress and Diabetic Complications

Many evidences from experiments have given link between diabetes and oxidative stress by measuring various biomarkers that include DNA damage biomarkers and lipid peroxidation products. It is believed that in the onset and progression of late diabetic complication, free radicals have got a major role due to their ability to damage lipids, proteins and DNA (Ayepola, Brooks & Oguntibeju, 2014). A variety of pathological conditions are induced by oxidative stress such as Rheumatoid arthritis, Diabetes mellitus and cancer (El Faramawy & Rizk, 2011). Free radical and oxidative stress induced complications from DM include coronary artery disease, Neuropathy, nephropathy, retinopathy (Phillips, Cataneo, Cheema & Greenberg, 2004) and stroke (Asfandiyarova, Kolcheva, Ryazantsev & Ryazantsev, 2007). In-vivo studies support the role of hyperglycaemia in the generation of oxidative stress leading to endothelial dysfunction in blood vessels of diabetic patients (Ceriello, 2006). Increase in the levels of glucose and insulin along with dyslipidemia in patients suffering from diabetes develops macroangiopathies that cause oxidative stress leading to atherosclerosis (Giugliano, Ceriello & Paolisso, 1995).

2.4.3 Biomarkers of Oxidative Stress in Diabetes Mellitus

Proteins

ROS reacts with some amino acid invitro, producing anything from modified, denatured and non-functioning proteins that in further may be responsible for oxidative stress (Nishigaki, Hagihara, Tsunekawa, Maseki & Yagi, 1981) Diabetic hyperglycaemia, by the process of free radical production, causes protein glycation and oxidative degeneration. The degree of such protein glycation is estimated by using some biomarkers such as glycated hemoglobin and fructosamine levels. Alteration in function and structure of antioxidant protein enzymes may also be due to nonenzymatic glycation such that detoxification of free radicals is effected enhancing oxidative stress in diabetes (Maritim *et al.,* 2003) According to invitro studies myeloperoxidase catalyzes the conversion of l-tyrosine to 3,3-dityrosine which serves as a crosslink between polypeptide chains of the same or different proteins making it a convenient biomarker for protein oxidation (Ylä-Herttuala, 1999).

Lipids

Diabetes mellitus produces disturbances in the lipid profile of body making the cells more susceptible to lipid peroxidation (Patricia, 2009). Experimental studies show that polyunsaturated fatty acids in cell membrane are extremely prone to attack by free radicals due to the presence of multiple bonds (Butterfiel, 1998). Lipid hyperperoxides (LHP) through intermediate radical reactions produce such fatty acids that generate highly reactive and toxic lipid radicals that form new LHP (Matough, Budin, Hamid, Alwahaibi & Mohamed, 2012). A critical biomarker of oxidative stress is Lipid peroxidation which is the most explored area of research when it comes to ROS (Hatice, 2004). Malondialdehyde (MDA) is formed as a result of lipid peroxidation that can be used to measure lipid peroxides after reacting it with thiobarbituric acid (Esterbauer, Schaur & Zollner, 1991).

Vitamins

Vitamins are very important part of biological system as they play important role in different biochemical processes. Among such vitamins, Vitamin A, C and E act as antioxidants by detoxifying the free radicals. Any alteration in their levels is significant biomarkers of oxidative stress. These vitamins also promote toxicity by producing pro-oxidants in certain conditions. Body levels of vitamin E have been reported to be either increased or decreased by diabetes. However conflicting reports present the deleterious effects of vitamin E on diabetes induced vascular changes (Maritim *et al.,* 2003).

Glutathione

Diabetes induces alterations in activity of enzymes glutathione peroxidase and glutathione reductase. These enzymes are found in cell that metabolizes peroxide to water and converting glutathione disulfide back into glutathione (Maritim *et al.,* 2003). Any alteration in their levels will make the cells prone to oxidative stress and hence cell injury.

In conclusion, oxidative stress has been demonstrated in many studies to participate in the progression of diabetes which plays important role during diabetes, including impairment of insulin action and elevation of the complication incidence. Antioxidants have already shown to be prospective in the treatment of diabetes both type 1 and type 2. Increase in the levels of oxygen and nitrogen free radicals (ROS/RNS) has been linked with lipid peroxidation, non-enzymatic glycation of proteins and oxidation of glucose which contributes toward diabetes mellitus and its complications. Most of the studies have shown relationship between oxidative stress and diabetes along with their complications related to heart, liver, kidney and eye. Thus, oxidative stress seems to be more worrying in metabolic disorders especially in type 2diabetes.

2.5 Lipid Profile and Diabetes Mellitus

Hyperglycaemia affects biochemical parameters and influences the progression of coronary heart disease and mortality rates in diabetic patients. Aggressive treatment to control hyperglycaemia is much more effective in reducing the number of complications than standard treatment (Van der does *et al.,* 1998; Herman, 1999).

The term hyperlipidaemia refers to an increase in concentration of one or more plasma or serum lipids, usually cholesterol and triglycerides and the term dyslipidaemia is used for either an increase or decrease in concentration of one or more plasma or serum lipids. Type 2 diabetic patients have markedly increased risk of coronary heart disease than similarly dyslipidaemic non-diabetic subjects (Bloomgarden, 1998).

Most recently, results of the Strong Heart Study indicate that LDL cholesterol is an independent predictor of cardiovascular disease in patients with diabetes, along with age, albuminuria, fibrinogen, HDL cholesterol (inverse predictor), and percent body fat (inverse predictor) (Van der does *et al.,* 1998). Atherogenic dyslipidaemia (diabetic dyslipidaemia) is characterized by 3 lipoprotein abnormalities: elevated very-low-density lipoproteins (VLDL), small LDL particles, and low high-density lipoprotein (HDL) cholesterol (the lipid triad) (Herman, 1999; Bunn, 1981).

Patients with diabetes can have many lipid abnormalities, including elevated levels of very low-density lipoprotein cholesterol (VLDL-C), low-density lipoprotein cholesterol (LDL-C), and triglycerides; and low levels of high-density lipoprotein cholesterol (HDL-C) (Haffner, 1998). These patients have a preponderance of abnormalities in the composition of LDL-C (smaller, denser particles), which increase atherogenicity even if the absolute concentration of LDL-C is not significantly increased. The combination of elevated levels of small, dense LDL-C particles and high triglyceride levels represents a lethal cholesterol abnormality known as pattern B.

About 20% of patients with type 2 diabetes have hypertriglyceridaemia or low HDL-C levels (Laakso, 1997). These abnormalities, as well as high VLDL-C levels and high total and VLDL-related triglyceride levels, are powerful risk indicators for CAD in patients with type 2 diabetes. In addition, the combination of elevated total cholesterol level, hypertension, and uncontrolled hyperglycaemia is implicated in the development of nephropathy.

In a study done in Russia, dyslipadaemia was detected in 84% diabetic patients (Kiriakov, Koev & Kavrykova, 1990). In one European study 40% were hyperlipidaemic according to the criteria of National cholesterol education programme, (cholesterol and triglyceride greater than 200 mg/dl). An additional 23% showed hypertriglyceridaemia (Stern, Patterson, Haffner, Hazuda & Mitchell, 1989). In another study hyperlipidaemia was found in 28% of diabetic patients (Paterson, Pettigrew, Dominiczak & Small, 1991).

Winocour *et al.,* has shown 40% hyperlipidaemia in IDDM patients. Hypertriglyceridaemia was found predominantly in all the cases while a few had combined hyperlipidaemia, pure hypercholesterolaemia however was not observed in a single case (Winocour, Durrington, Ishola, Hillier & Anderson, 1989).

Hypertriglyceridaemia, which is comparatively more common in NIDDM than IDDM (Garg and Grundy, 1990) is probably due to increased production and reduced clearance of rich lipoproteins-VLDL (Hirano, Mamo, Takeuchi, Nagano & Takahashi, 1991). It is further interesting to note that even the 1st degree relatives of NIDDM cases have dyslipidaemia in general and hypertriglyceridaemia in particular (Laws, Stefanick & Reaven, 1989).

The poorer the glycaemic control, the higher the degree of hypertriglyceridaemia. This relationship can be explained by the glucoregulatory and lipolytic actions of insulin, and defect in this can lead to dyslipidaemia more so in NIDDM patients (Baynes *et al.,* 1991). If good glycaemic control is achieved then not only the size of VLDL particle is reduced but also increases in the concentration of apo - B protein fraction of VLDL takes place. These

changes in turn lead to increased clearance of VLDL particles and ultimately dislipedaemia reverts (Leowsky, James, Taton & Pometta, 1988).

2.6 Phenolic Compounds

The term 'phenolic' or 'polyphenol' can be defined chemically as a substance which possesses an aromatic ring bearing one or more hydroxy substituents, including functional derivatives (esters, methyl ethers, glycosides etc.) (Harborne, 1989). Most phenolics have two or more hydroxyl groups and are bioactive substances occurring widely in food plants that are eaten regularly by substantial numbers of people.

2.6.1 Sources of Phenolic Compounds

Phenolic compounds are a group of chemical compounds that are widely distributed in nature.

They are simple compounds present in most fresh fruits and vegetables, or complex compounds present in bark, roots and leaves of plants. A group of polyphenols, responsible for the color of many fruits, vegetables, and flowers, are known as anthocyanins.

Plants synthesize a vast range of secondary metabolites with a significant portion consisting of phenolic compounds and flavonoid compounds (Crozier, Ashihara & Clifford, 2006). These phytochemicals are structurally diverse, and many are distributed among a very limited number of species within the plant kingdom.

This character allows them to act as biodiagnostic markers in chemotaxonomic studies. Phenolic compounds and flavonoids accumulate in relatively high amounts in plants and appear to have a myriad of supplemental functions in a plant’s life cycle. These include structural roles in different supporting or protective tissues, involvement in defense strategies, as attractants for pollinators and seed-dispersing animals, and as allelopathic agents, ultra violet (UV) protectants and signal molecules in the interactions between plants and their environment. One of the most versatile classes of flavonoids, the anthocyanins, protect chloroplasts from photodegradation by absorbing high-energy quanta, while scavenging free radicals and reactive oxygen species (ROS) (Gould, 2004).

Flavonols, as well as providing protection against the damaging effects of UV-B light, are also involved in promoting the growth of pollen tubes down the style to facilitate fertilization. In addition, isoflavonoids play important defense roles against pathogen and insect attack and are key signal molecules in the formation of nitrogen-fixing root nodules in legumes. After the death of plants, phenolic compounds may persist for weeks or months and affect decomposer organisms and decomposition processes in soils. Therefore, their effects are not

restricted to only the plant itself but may extend to the functioning of whole ecosystems (Horner, Gosz & Cates, 1988).

Secondary metabolites, other than providing plants with unique survival or adaptive strategies, are of commercial significance to mankind. They have been used as dyes, fibers, glues, oils, waxes, flavoring agents, drugs, and perfumes and are viewed as potential sources of new natural drugs, antibiotics, insecticides, and herbicides (Croteau, Kutchan & Lewis, 2000; Dewick, 2002). In recent years the role of phenolic compounds and flavonoids as protective dietary constituents has become an increasingly important area of human nutrition research.

Unlike the traditional vitamins, they are not essential for short-term well-being, but there is increasing evidence that modest long-term intakes may exhibit a potential for modulating human metabolism in a manner favorable for the prevention or reduction in the risk of degenerative diseases such as cardiovascular diseases, diabetes, obesity, and cancer (Anderson, Anthony, Cline, Washburn & Garner, 1999).

2.6.2 Types and Properties of Phenolic Compounds

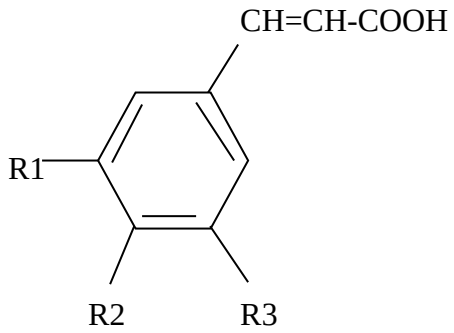
The phenolic compounds which occur commonly in food material may be classified into three groups, namely, simple phenols and phenolic acids, hydroxycinnamic acid derivatives and flavonoids.

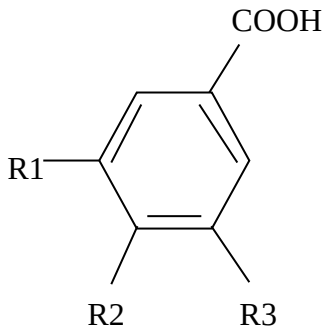
Volatile Phenols

Simple phenols such as phenol, o-cresol, 4-ethylphenol, guaiacol, 4-vinylguaiacol and eugenol have been found in the volatiles of fruits and vegetables (Tatum, Nagy & Berry, 1975; Buttery, Guadgni, Ling, Seifert & Lipton, 1976). The 4-vinylguaiacol is a major flavor component of citrus fruits and contributes a rottenî flavor to orange juice (Tatum *et al.*, 1975). The 4-ethylguaiacol, eugenol, and 4-ethylphenol, a group of odorants with high flavor dilution (FD) factors, are a key difference between young and aged red wines (Margarita, Ricardo, Juan & Vicente, 2001).

Phenolic Acids

Hydroxybenzoic and hydroxycinnamic acids are predominant phenolic acids found in plants. Differences between their derivatives consist in the different patterns of hydroxylations and methoxylations of their aromatic rings. The structures of some of these compounds are shown in Figures 1.





R1	R2	R3	Compound
H	OH	H	*p*-Hydroxybenzoic acid
OH	OH	OH	Gallic acid
H	OH	OH	Protocatechuic acid

(a)

R1	R2	R3	Compound
H	OH	OH	Caffeic acid
CH_3O	OH	OH	Ferulic acid
H	OH	H	*p*-Coumaric acid

(b)

Figure 1: Basic structure of (a) hydroxybenzoic and (b) hydroxycinnamic acid derivatives

Source: Schuster and Hemann (1985)

a. Hydroxybenzoic Acids

Hydroxybenzoic acids have a general structure of C6-C1 (Figure 1a). Hydroxybenzoic acids are commonly present in bound form. They are components of complex structures such as hydrolyzable tannins and lignins. Hydroxybenzoic acids are also found in the form of sugar derivatives (Schuster and Hemann, 1985). The hydroxybenzoic acid content in foods of plant origin is generally low. Gallic acid is one of the common hydroxybenzoic acids. Its dimeric condensation product and related dilactone, ellagic acid, are commonly found in plants. There is a particular interest in ellagic acid in fruits because of the increasing evidence of its anticarcinogenic and antioxidant effects (Meyer, Heinonen & Frankel, 1998).

b. Hydroxycinnamic Acids

Hydroxycinnamic acids (Figure 1b) are also commonly found in foods of plant origin. *p*-Coumaric, caffeic, ferulic and sinapic acids are major hydroxycinnamic acids found in fruits. Among these, caffeic acid is the predominant hydroxycinnamic acid in many fruits. Caffeic acid represents over 75% of the total hydroxycinnamic acids in fruits. Caffeic acid has been found in plums, apples, apricots, blueberries and tomatoes (Kono, Shibata, Kodama, Ueda & Sawa, 1995).

Hydroxycinnamic acids are mainly present in bound form and are rarely found in free form. Hydroxycinnamic acids usually occur in various conjugated forms. The conjugated forms are esters of hydroxyacids such as quinic, shikimic and tartaric acid, and their sugar derivatives. The free hydroxycinnamic acids can be released from chemical or enzymatic hydrolysis during tissue extraction (Schuster and Hemann, 1985).

Flavonoids

Flavonoids represent the most common and widely distributed group of plant phenolics. Their common structure (C6-C3-C6) consists of two aromatic rings (A ring and B ring) linked through a three carbon bridge that is usually an oxygenated heterocycle (C ring). Figure 2 shows the basic structure and the system used for the carbon numbering of the flavonoid nucleus. The major flavonoid classes include anthocyanidins, chalcones, flavanols, flavanones, flavones, flavonol, and isoflavones.

The variability of the flavonoids is based on the hydroxylation of the pyrone ring, absence or presence of double bond, the number of hydroxyls in the A ring and B ring, and/or a double bonded oxygen atom attached to position 4 of the C ring. Flavonoids may be monomeric, dimeric, or oligomeric. Polymeric flavonoids, known as tannins, are divided into two groups, condensed and hydrolysable. Condensed tannins are polymers of flavonoids while hydrolysable tannins contain gallic acid.

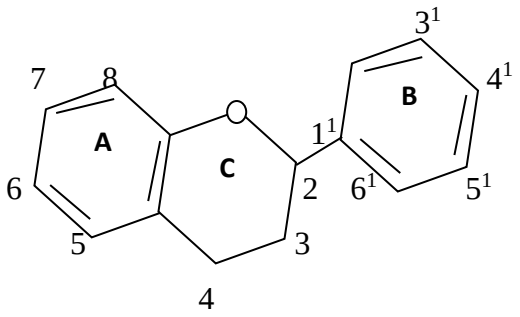
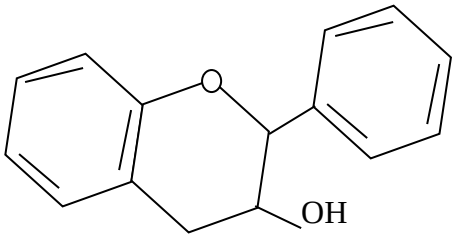


Figure 2: Basic structure of flavonoid skeleton

Source: Pietta (2000)

a. Flavanols and Flavonols

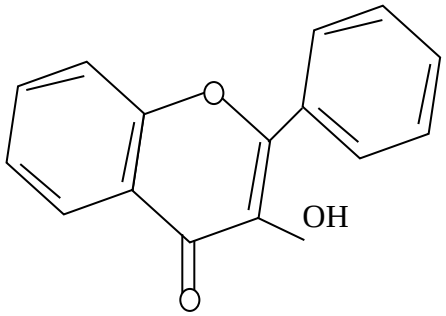
Flavanols are known as flavan-3-ols (Figure 3a), and they are the subunits of proanthocyanidins, which have a hydroxyl group attached to the 3 position of the C ring, no positive charge on the oxygen atom and no double bond in the C ring. The structures of flavonols (Figure 3b) are very similar to those of flavanols, except that there is a double-bonded oxygen atom attached to position 4 of the C ring and a double bond in the C ring.



Positions 5, 7, 3´, 4´: OH →Catechin

Positions 5, 7, 3´, 4´, 5´: OH→Epicatechin

(a)



Positions 5, 7, 4´: OH →Kaempferol

Positions 5, 7, 3´, 4´: OH→Quercetin

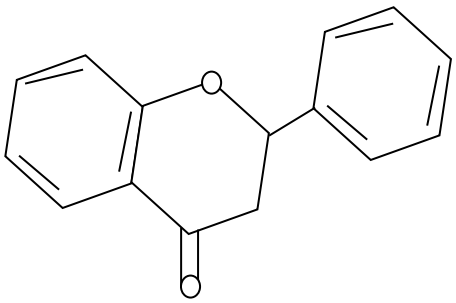
(b)

Figure 3: Basic structure of (a) flavan-3-ol and (b) flavonol skeletons

Source: Pietta (2000)

b. Flavanones and Flavones

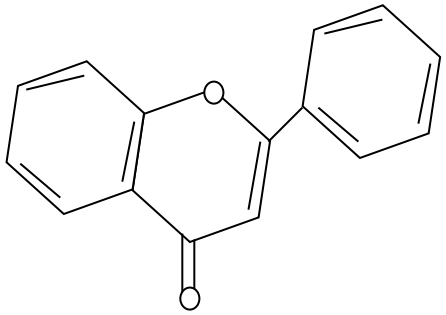
Flavanones (Figure 4a) and flavones (Figure 4b) have structures similar to those of flavanols and flavonols, respectively. But, in each case, there is no longer a hydroxyl group attached to the 3 position of the C ring.



Positions 5, 7, 4´: OH →Naringenin

Positions 5, 7, 3´, 4´: OH→Eriodictyol

(a)



Positions 5, 7, 4´: OH →Apigenin

Positions 5, 7, 3´, 4´: OH→Luteolin

(b)

Figure 4: Basic structure of (a) flavanone and (b) flavone skeleton

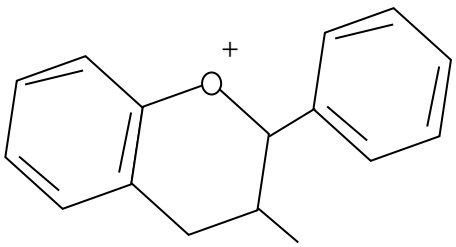
Source: Pietta (2000)

c. Anthocyanins

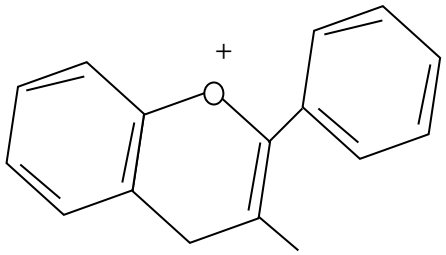
Anthocyanins are widely distributed among fruits and vegetables. They are one of the main classes of flavanoids. They contribute significantly to the antioxidant activities of the

flavanoids (Lapidot, Harel, Akiri, Granit & Kanner, 1999). Anthocyanins are water soluble pigments responsible for red, blue and violet colours.

Anthocyanins (Figure 5a) are glycosylated anthocyanidins with sugars generally attached to the 3-hydroxyl position of the anthocyanidin (Figure 5b). In some cases the sugar residues are acylated by p-hydroxybenzoic, p-coumaric, caffeic, ferulic, sinapic, acetic acid, oxalic acid, malic acid, or succinic acid. Anthocyanidin is an aglycone. This means that there is no sugar group or other functional group attached to the flavan nucleus. Also, the oxygen atom on the C ring has a positive charge on it, and there are two double bonds in the C ring. In addition to hydroxylated anthocyanidins, such as delphinidin, cyanidin, and pelargonidin, there are also methylated anthocyanidins (malvidin, peonidin, and petunidin).



(a)



Positions 5, 7, 4′: OH →Pelargonidin

Positions 5, 7, 3′, 4′: OH→Cyanidin

(b)

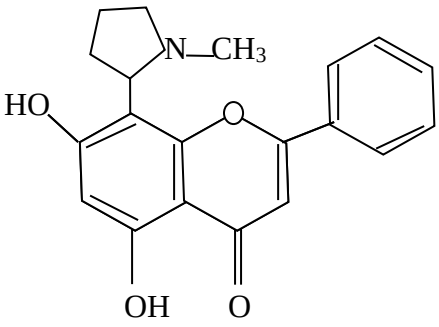
Figure 5: Basic structure of (a) anthocyanin and (b) anthocyanidin skeletons

Source: Pietta (2000)

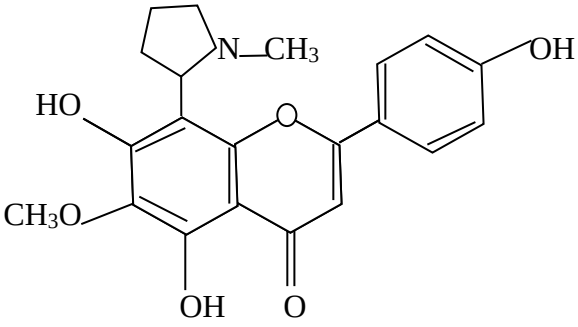
d. Flavanoidal Alkaloid

Flavanoid alkaloids are of interest because of the biological activities which they have been found to possess. These include antiviral properties and tyrosine kinase inhibition, the latter being the basis of their potential use in treating inflammatory conditions and acting as anti-neoplastic agents.

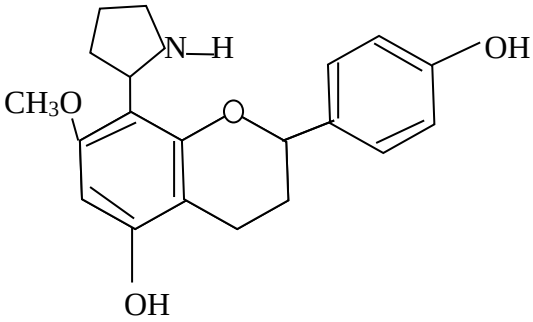
The classification of flavanoidal alkaloids is somewhat unusual since most alkaloids are classified chemically according to the nitrogen-containing ring system. However, the class under consideration is typified by part of the molecule to which the nitrogenous moiety is attached (Houghton, 2002). Some naturally occurring flavonoidal alkaloids are shown in Figure 2.6.



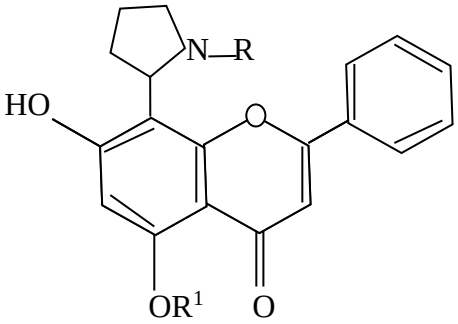
(1) Ficine



(2) Phyllospadine



(3) Vochysine

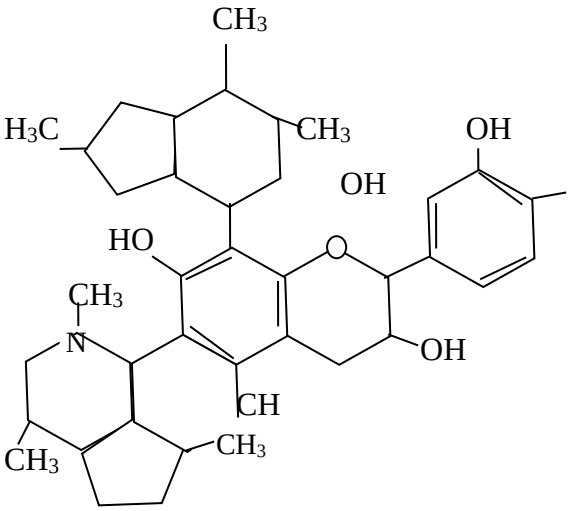


R R´

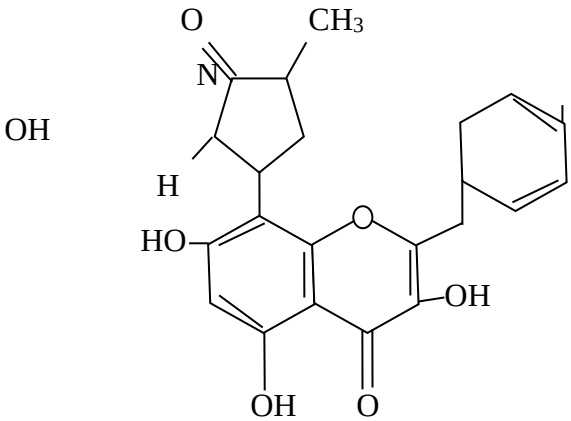
(4) CH3 CH3 Buchenavianine

(5) CH3 H3 *O*-Demethylbuchenavianine

(6) H H N, *O*-Didemethylbuchenavianine



(7) Kopsirachine



(8) Lilianine

Figure 2.6: Some structures of flavonoidal alkaloids

Source: Houghton (2002)

2.6.3 Health Benefits and Mode of Action of Phenolic Compounds

The rapid rise of degenerative diseases worldwide is threatening economic and social development as well as the lives and health of millions of people. It represents a major health challenge to global development in the coming century.

It is estimated that up to 80% of cardiovascular disease, 90% of Type II diabetes, and one third of cancers can be avoided by changing lifestyle, including diet (World Health Organisation [WHO], 2003). Diet-related high cholesterol, high blood pressure, obesity, and insufficient consumption of fruits and vegetables have been cited as significant interlinking risk factors that cause the majority of these diseases.

There is, therefore, increasing interest in the role of nutrition and specific dietary constituents in the prevention of such diseases. Flavonoids and phenolic compounds are prominent among dietary constituents that are the focus of such interest.

Since the 1990s a number of epidemiological studies have been carried out attempting to correlate high dietary phenolic compounds and flavonoid intake, through the consumption of fruits and vegetables, with reduced risk of degenerative diseases. Many, but not all, of these studies have indicated some degree of inverse associations between high dietary phenolic/flavonoid intake and reduction of degenerative diseases (Steinmetz and Potter, 1996; Law and Morris, 1998; Riboli and Norat, 2003). Since oxidative stress imposed by ROS is known to play a crucial role in the pathophysiology associated with neoplasia, atherosclerosis, and neurodegenerative diseases, the potential mechanism of the protective effects of phenolic compounds and flavonoids were thought to be due to direct scavenging of free radicals (Heim, Tagliaferro & Bobilya, 2002).

Accumulating evidence now indicates the importance of interactions between various phytochemicals in reducing the risk of various degenerative diseases (Chan, Mattiacci, Hwang & Shah, 2000; Mouria *et al.*, 2002; Mertens-Talcott, Talcott & Percival, 2003).

The combination of antioxidative agents with different modes of action is thought to increase efficacy and minimize toxicity. The abilities of phenolic-based antioxidant therapies to decrease ROS levels has been shown to produce the best health benefits through a diet rich in multiple antioxidants rather than a high dosage of a single supplement (Lee and Lee, 2006). Evidence of the potential benefits of food synergy was provided by Liu *et al* (2000) when they demonstrated that a combination of fruits, such as orange, apple, grape, and blueberry, displayed a synergistic effect on antioxidant activity invitro. The median effective dose (EC 50) of each fruit in combination was five times lower than the EC50 of each fruit alone,

suggesting synergistic effects due to the combination of the four fruits. In another study, Sakamoto (2000) emphasized the importance of consuming black tea together with soybean products as commonly occurs in a typical Japanese diet. In this study, the arubigen in black tea did not alter the invitro growth of human prostate cancer cells. However, a small amount of thearubigen (0.5mgmL–1) administered with genistein (20mgmL–1), the major isoflavone in soybean, synergistically inhibited cell growth and increased the DNA distribution at the G2 M phase of the cell division cycle by 34% compared with genistein alone (Sakamoto, 2000). Similar conclusions were reached by Temple and Gladwin (2003) when they reviewed 200 cohort and case–control studies that provided risk ratios concerning intake of fruits and vegetables and risk of cancer. Their studies showed that the cancer- preventing action of fruits and vegetables is most probably due to the many bioactive compounds that act in concert to prevent cancer rather than being due to one or two potent anticarcinogens.

Nutrients generally have very specific functions such as being an enzyme cofactor. In contrast, in addition to their additive and synergistic effects, phenolic compounds and flavonoids, often exhibit pleiotropic effects that in combination may reduce the risk of chronic disease. For instance, curcumin, the active constituent of turmeric (Curcuma longa), a root vegetable, has been shown to be beneficial in all three stages of carcinogenesis (Thangapazham, Sharma & Maheshwari, 2006). Isoflavones, the bioactive ingredient in leguminous vegetables, not only cause a small reduction in blood cholesterol but also reduce blood pressure, arterial dimensions, and oxidative stress (Anderson *et al.,* 1999).

This combined effect may cause a reduction in the risk of coronary heart disease (Kris-Etherton *et al.,* 2004).

In addition to the complexity mentioned above, the health implications of dietary phenolic compounds and flavonoids are also dependent on the composition of the components of the diet and the bioavailability of the individual compounds under study. Accumulating evidence on the absorption and bioavailability of phenolic compounds and flavonoids in humans reveals that most of these phytochemicals are modified during absorption from the small intestine, through conjugation and metabolism, and by the large intestine, mainly through the actions of the colonic microflora, and by subsequent hepatic metabolism (Graefe *et al.,* 2001; Manach, Scalbert, Morand, Remesy & Jimenez, 2004; Jaganath, Mullen, Edwards & Crozier, 2006). Thus, metabolites that reach the cells and tissues are chemically, and, in many instances, functionally distinct from the dietary form, and such features underlie their bioactivity (Kroon *et al.,* 2004). This, in addition to the fact that in most instances very low levels of dietary phenolic compounds and flavonoids are actually absorbed and appear in the

bloodstream (10mM), implies that the concept of these compounds functioning as hydrogen-donating antioxidants in vivo appear to be an over- simplified view of their mode of action (Williams, Spencer & Rice-Evans, 2004; Williamson and Manach, 2005; Fraga, 2007).

It has been hypothesized that cells respond to phytochemicals through direct interactions with receptors or enzymes involved in signal transduction, or through modifying gene expressions that may result in alteration of the redox status of the cell that may trigger a series of redox-dependent reactions (Williams *et al.,* 2004). There is now emerging evidence that flavonoids may play an important role in molecular processes especially as modulators of intracellular signaling cascades, which are vital to cellular function (Williams *et al.,* 2004). For example, in a recent study carried out by Mackenzie *et al.* (2008), a naturally occurring phenolic compound, curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione,1) was found to deregulate signaling cascades, such as NF-kB, leading to a decreased expression of proteins involved in cell proliferation and apoptosis. In another study on Caco-2 cells, hexameric procyanidins was found to inhibit TNFa-induced NF-kB activation, which is believed to play a central role in inflammation including human intestinal bowel disease (Erlejman, Jaggers, Fraga & Oteiza, 2008).

There is growing evidence from human feeding studies that the absorption and bioavailability and thus bioactivity of phenolic compounds and flavonoids are very much dependent on the nature of their chemical structure.

2.7 *Zanthoxylum zanthoxyloides*

Zanthoxylum zanthoxyloides, also known as *Fagara zanthoxyloides*, the Senegal prickly-ash or artar root, is a plant specie in the genus *Zanthoxylum* and an indigenous plant used widely as chewing stick for tooth cleaning in West Africa (Adebiyi *et al.,* 2009; Adegbolagun and Olukemi, 2010).

The plant contains isomeric divanilloylquinic acids (3,4-O-divanilloylquinic acid or burkinabin A, 3,5-O-divanilloylquinic acid or burkinabin B and 4,5-O-divanilloylquinic acid or burkinabin C) (Ouattara *et al.,* 2009). Burkinabin C, a type of hydrolysable tannin can be found in the root bark of *F. zanthoxyloides* (Ouattara *et al.,* 2004). It also contains fagaronine, a benzophenanthridine alkaloid (Dupont *et al.,* 2005).

Several studies on the various effects of its extracts have been reported. For example, Kassim *et al* (2005) reported the anti-malarial activity attributed to benzophenanthridine alkaloid, fagaronine from *F. zanthoxyloides'* root extracts. Anti-malarial activity was also reported in a study using extracts from trunk barks of *F. zanthoxyloides* (Gansane, Sanon, Ouattara, Hutter

& Ollivier, 2010). On the other hand, Patel, Das, Prakash & Yasir (2010) named the compound nitidine as the agent in *F. zanthoxyloides'* anticancer activity while an anti-inflammatory property due to ortho-hydroxymethyl benzoic acid made *F. zanthoxyloides* useful in the management of pain in sickle cell crisis (Oyedapo and Famurewa, 1995; Folasade, Olukemi & Jones, 2006). More recently, the potential of *Z. zanthoxyloides* leaf, bark and root extracts as a biopesticide for stored food protection has been reported (Udo, 2011).

A study by Williams, Soelberg and Jäger (2016) showed that ethanolic extracts of *Z. zanthoxyloides* have *invitro* anthelmintic properties against the nematode *Ascaris suum*, a swine parasite that is closely related to the human parasite *A. lumbricoides*. The half maximal effective concentration (EC50) values were 94 μg/mL and 132 μg/mL, for roots and root bark, respectively. The authors concluded that these results encourage further investigation of the use of this plant as complementary treatment options for ascariasis (Williams *et al.*, 2016).

2.7.1 Botanical Information

Scientific Classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Rosids
Order:	Rutales
Family:	Rutaceae
Subfamily:	Toddalioideae
Genus:	*Zanthoxylum*
Species:	***Z. zanthoxyloides***

Binomial name
Zanthoxylum zanthoxyloides (Lam.)

Source: Theplantlist.org (2013)

Zanthoxylum is pantropical and comprises about 200 species, with tropical America being richest in species. Mainland Africa harbours about 35 species, whereas about 5

species are common in Madagascar. The orthographic variation *Zanthoxylum xanthoxyloides* is common. Several other *Zanthoxylum* species are medicinally used in West Africa. *Zanthoxylum atchoum* is common in Côte d'Ivoire. The strong-smelling roots are sniffed as an emmenagogue. It is a good bee-plant as the flowers produce large quantities of nectar.

Zanthoxylum chevalieri (synonym: *Fagara pubescens* A.Chev.) occurs in Guinea east to Ghana. In western Côte d'Ivoire, powdered leaves are used as a snuff to treat migraine.

Zanthoxylum viride (A.Chev.) occurs in Guinea East to Cameroon. In Côte d'Ivoire a leaf decoction is used as a mouth-wash to soothe toothache or leaves are chewed. Pulped leaves are also applied to leprous sores, and a bark decoction is taken orally. Crushed root bark is applied as an enema to treat gonorrhoea. Pulped roots are used in embrocation to treat head-ache, rheumatic, lumbar and intercostal pain. Bark macerated in wine or a bark decoction is drunk as an aphrodisiac and to treat venereal diseases. A bark decoction is also taken to treat intestinal worms and dysentery. Crushed bark is applied to scabies. The wood can be used in cabinet work, although the pieces are usually small.

2.7.2 Description

Shrub or small tree, spiny and more or less scandent, up to 6–8(–12) m tall, with straight, often short bole and rounded and quite dense crown; bark grey to beige, rough, with fine vertical fissures, often with woody prickle-bearing protuberances; slash yellow, odorous, orange-mottled beneath; stems glabrous, grey, with solitary prickles. Leaves alternate, glabrous, imparipinnately compound with 5–7(–11) opposite or alternate leaflets, up to 12(–20) cm long; petiole 2–5 cm long, glabrous, spiny beneath with recurved prickles; stipules absent; petiolules 2–5 mm long; leaflets obovate to elliptical, 5–10 cm × 2–4 cm, base cuneate to rounded, apex obtuse or rounded, sometimes apiculate or notched, with many glandular dots, smelling of pepper and lemon when crushed, rigidly papery, pinnately veined with 10–14 pairs of lateral veins, barely prominent, fusing near the margin. Inflorescence a lax terminal or axillary panicle 5–25 cm long, with short branches. Flowers unisexual, regular, 5-merous, white or greenish, sessile; corolla barely open; male flowers with stamens slightly exserted; female flower with superior ovary, 1-celled, style short, lateral. Fruit an ovoid follicle, 5–6 mm in diameter, brown, with glandular dots, dehiscent, 1-seeded. Seed black to bluish, shiny, long persistent in the fruit.

2.7.3 Properties

Zanthoxylum zanthoxyloides contains a high diversity of essential oils and alkaloids, as well as several aliphatic and aromatic amides. Analysis of the essential oil obtained from the fruits collected from different localities showed the existence of several chemovariants. The essential oil from one sample of fruits from Cameroon contained monoterpenes as main components: α-pinene (38.2%), trans-β-ocimene (5.4%), citronellol (3.3%), sabinene (3.2%), myrcene (3.1%), limonene (3.0%), citronellyl acetate (3.0%), α-terpinolene (2.7%), α-phyllandrene (2.6%), geraniol (1.9%), terpinen-4-ol (1.5%), p-cymene (1.2%), methyl citronellate (1.2%) and β-pinene (1.2%). Another sample contained as main components: β-citronellol (18.1%), geraniol (16.2%), 2,6-dimethyl-2,6-octadiene (9.3%), geranyl acetate (5.9%), isopulegol (5.4%), D-limonene (4.8%), β-citronellal (4.7%) and the sesquiterpene manoyl oxide (5.5%). The essential oil from a fruit sample from Benin contained mainly monoterpenoids, with β-ocimene (41.5%), linalool (11.3%) and geranial (9.5%) as main components. The leaf essential oil only contained monoterpene hydrocarbons (98.2%), mainly β-ocimene (31.9%), α-pinene (26.5%) and myrcene (30%).

The stem and root barks contain benzophenanthridine, furoquinoline and aporphine alkaloids. From the root bark the benzophenanthridines fagaronine, dihydroavicine, chelerythrine and oxychelerythrine were isolated, and from the stem bark fagaronine and chelerythrine. From the root bark the furoquinolines skimmianine and 8-methoxydictamine, as well as the aporphines magnoflorine, berberine, tembetarine and N-methyl-corydine were isolated. From the roots, fruits and stem bark several aliphatic amides have been isolated, including the pungent N-isobutyldeca-2, 4-dienamide and N-isobutylocta-2,4-dienamide, which are main components of pellitorine. The root bark also contains the aromatic amides arnottianamide, fagaramide, piperlonguminine, rubemamin and N-isopentyl-cinnamamide. From the stem bark many coumarins were isolated: umbelliferone, scopoletin, scoparone, xanthotoxin, imperatorin, bergapten, marmesin and pimpinellin; the root bark and the stem bark contain the lignan sesamin, while the root bark also contains its C-7 epimer asarinin. From the aerial parts and roots the sterols zanthoxylol, diosmin, fagarol and hesperidin have been identified, as well as triterpenes such as lupeol, β-sitosterol, stigmasterol, campesterol and β-amyrin. From the roots a series of acids was isolated: vanillic acid, hydroxybenzoic acid, parahydroxybenzoic acid, 2-hydroxymethyl benzoic acid and parafluorobenzoic acid as

well as the divanilloylquinic acids burkinabin A, B and C.

Crude root bark extracts showed significant anti-inflammatory and analgesic activities in rodents. They also showed anti-sickling activity in several small clinical trials; the isolated acids also showed significant anti-sickling activity. Different plant extracts showed low toxicity in laboratory tests. Crude root bark extracts showed moderate antibacterial activity invitro against a range of pathogenic bacteria. The essential oil of the fruits showed moderate to significant antibacterial activity. Ethanolic leaf extracts showed low antifungal invitro, whereas root and stem bark extracts showed moderate to good antifungal activity invitro. The alkaloid extract of the stem bark showed significant antiplasmodial activity invitro. Different root bark extracts showed moderate antiviral activity against herpes simplex virus and antiprotozoal activity against *Leishmania major*. Extracts of different plant parts as well as powdered plant parts showed significant insecticidal and antifeedant activities against a range of crop pests. The leaves fed to rabbits showed anthelmintic activity against *Ascaris lumbricoides*, as well as significant anthelmintic activity against *Haemonchus contortus* when fed to sheep. An ethanolic root bark extract exhibited moderate antioxidant activities invitro.

Pellitorine showed insecticidal, antibacterial and anti-sickling activity invitro. The benzophenanthridine alkaloid fagaronine demonstrated strong antileukemic activity against both L-1210 and P-388 leukemia cell lines as well as anti-sickling properties. Other benzophenanthridine alkaloids such as chelerythrine and berberine also showed antimicrobial activities. However, the toxicity of benzophenanthridine alkaloids prevents their clinical application. Several coumarins isolated from *Zanthoxylum zanthoxyloides* showed antifungal activities. Scoparone and scopoletin have demonstrated anticonvulsive effects in experimental animals. β-Sitosterol showed significant anti-inflammatory activity when administered intraperitoneally to mice and rats and was orally effective against carrageenin-induced oedema. β-Sitosterol also exhibited anti-pyretic activity.

The mineral composition of the fruit pericarp per 100 g is: Ca 90 mg, P 41 mg, Fe 2 mg, Na 10 mg, K 46 mg, Mg 52 mg and Cu 55 mg.

2.7.4 Uses

Medicinal Uses

Throughout West Africa the aromatic roots, stem bark and leaves are commonly used in traditional medicine. They are considered antiseptic, analgesic and diaphoretic. Root or

stem bark macerations, decoctions or infusions are widely taken to treat malaria, fever, sickle cell anaemia, tuberculosis, paralysis, oedema and general body weakness. They are also widely taken to treat intestinal problems, including colic, dysentery, intestinal worms, gonorrhoea and urethritis, also as an emmenagogue, stimulant and to treat pain during childbirth, migraine and neuralgia. The roots are externally applied to ulcers, swellings, haemorrhoids, abscesses, snake bites, yaws, wounds, leprosy, and syphilitic sores as well as rheumatic and arthritic pain, and hernia.

The roots and stem bark give a warm, pungent and benumbing effect on the palate when chewed, and are widely used in the treatment of sore gums, toothache and dental caries. A decoction of the roots is used as a mouthwash and against a sore throat. In Côte d'Ivoire sap from the pulped bark is applied as eye drops to treat eye infections, notably conjunctivitis with pus. In Ghana root and stem bark powder is taken to treat whooping cough.

In southern Nigeria a decoction of the stem bark and roots is taken to treat cancer.

Other Uses

In southern Nigeria, pulped stem bark and root bark is thrown in the water to stupefy fish.

In West Africa, it is planted as a hedge, as the thorns make it impenetrable. Sheep browse the leaves. The wood is used for manufacturing of torches. The timber is yellow, very hard and termite-resistant and used for building purposes, including poles and posts. It also makes good firewood. The roots, young shoots and twigs are commonly used as chew-sticks. The bark or young branches contain much resin, which makes them suitable for ceremonial torches. The spines are thrown into fire to give off a scented smoke. The leaves, which smell like citronella, and the seeds, which taste strongly of cinnamon or pepper, are commonly used to season food. From the seeds, necklaces are made. *Zanthoxylum zanthoxyloides* also has numerous magico-religious uses, including protection against spirits. It also serves as fetish plant.

CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant Materials

Fresh leaves of *Z. zanthoxyloides* leaves were collected from Abakiliki, Ebonyi State, Nigeria. The plants were identified and authenticated by a taxonomist at the Federal University of Technology Owerri (FUTO).

3.1.2 Chemicals, Reagents, Kits and their Sources

Alloxan monohydrate, ethylacetate and methanol were manufactured by Sigma, Germany. All assays kits were from Randox laboratories Ltd. Ardmore, Co. Antrm UK. Chemicals and reagents used were of analytical grade unless otherwise stated. Folin Ciocalteu Phenol Reagent, Gallic Acid, Hydrogen Peroxide, 2,2-Diphenyl-2-picrylhydrazyl (Sigma-Aldrich), Sodium chloride, Sodium hydroxide, Sodium carbonate, Sodium bicarbonate, Acetic acid, Potassium dichromate, Potassium phosphate, Dipotassium phosphate,Trichoroacetic acid, Adrenalin, Thiobarbituric acid (Sigma-Aldrich) were all obtained from Okey Medicals Kaduna, Kaduna State.

3.1.3 Equipment

Rotary evaporators, Sherwood Colorimeter 257, Grant JB Series Water Bath, Heraeus Labofuge 300 Centrifuge, Gallenkamp Incubator, MicropH 2000, RS-232C Weighing Balance, Thomas-Wile Laboratory mill, what man paper.

3.1.4 Animals

Thirty adult male rats (Wistar) weighing between 100.0-195.0g were purchased from the Department of Veterinary Medicine, University of Nigeria, Nsukka. The animals were housed in standard animal cages, fed with commercial feed and water, *ad libitum*, and acclimatized for 7 days.

3.2 Methods

3.2.1 Preparation of Plant Extract

One thousand grammes (1000g) of dried and pulverized *Z. zanthoxyloides* leaves were macerated twice in 4.0 L of absolute methanol for 72 hours. Filtration was done using cheese cloth and afterwards, a Whatman No. 1 filter paper. Combined filtrates were evaporated using

rotary evaporator at 40^{o}C. The crude extracts obtained were dissolved with ethylacetate and water in a ratio of 2:1 respectively; water being denser was filtered out first using a thistle funnel followed by ethylacetate where the phenolic compounds were dissolved in. The filtrate was evaporated again using rotary evaporator at 40^{o}C which was then stored in the refrigerator and used for the experiments.

3.2.2 Determination of Phenolic Contents

Total phenolic content was determined using the method of Swain and Hillis as described by Wettashinghe and Shaidi (2000). 50mg of methanol extracts were dissolved in 100% methanol to obtain a concentration of 0.5 mg/ml (Solution A). Folin-Denis reagent (0.5 ml) was added to centrifuge tubes containing 0.5 ml of solution A. Tubes were shaken and 1ml of a saturated sodium carbonate solution added. Volume was then adjusted to 10ml by the addition of 8 ml of deionised water and the content was mixed vigorously. Tubes were allowed to stand at ambient temperature for 25 minutes and then centrifuged for 5 minutes at 4000 xg. Absorbance of the supernatants was measured at 725 nm. A blank sample (treated in the same way but without the extract) for each extract was used for background subtraction. Content of total phenolics in each extract was determined using a standard curve prepared for Tannic acid. Total extracted phenolic compound was expressed as mg/Tannic acid equivalent/g extract.

3.2.3 Determination of Flavonoid Contents

The total flavonoid content of Plant extract was determined colorimetrically as described by Zou, *et al* (2004). In brief, 0.5 ml of sample solution (A) above was mixed with 2.0 ml of distilled water and subsequently with 0.15 ml of 5% $NaNO_2$ solution. After 6 minutes of incubation, 0.15 ml of 10% $AlCl_3$ solution was added and then allowed to stand for 6 minutes, followed by addition of 2ml of 4% NaOH solution to the mixture. Immediately, water was added to the sample to bring the final volume to 5.0 ml. The mixture was thoroughly mixed and allowed to stand for another 15 minutes. The absorbance was read at 510 nm. The total flavonoid content was expressed in milligrams of Quercetin equivalent per gram of extract. Content of total flavonoids in each extract was determined using a standard curve prepared for Quercetin. Total extracted flavonoid was expressed as mg (+) Quercetin equivalent/g extract.

3.2.4 Grouping and Feeding of Animals

The rats were kept in cages which were cleaned of waste twice a day. They were exposed to 12 hours each of natural daylight and darkness and given rat chow and water *ad libitum.* They were divided into six groups; group A (normal control) not induced with diabetes, group B (diabetic/negative control) induced with diabetes but not treated, group C, D and E (diabetic test groups) were induced with diabetes and treated daily with 100mg/kg, 200mg/kg and 400mg/kg body weight *Z. zanthoxyloides* phenolic extracts respectively while group F was treated with 5mg/kg body weight of a standard diabetic drug (glibenclamide).

3.2.5 Induction of Diabetes and Treatment of Animals with Plant Extract

The thirty adult male rats were weighed and their base line blood sugar levels recorded using a glucometer (Accucheck Active). A single dose intraperitoneal injection of alloxan monohydrate (2, 4, 5, 6 tetraoxypyridine 5, 6-dioxyuracil) in 5 percent solution, a dose of 130 mg/kg body weight was given to twenty-five rats, whereas five rats served as the normal control group. Three days after alloxan administration, the blood sugar levels of the rats were recorded and the diabetic rats (with blood sugar levels $\geq$ 250.0 mg/dl) were divided into five groups of five animals each according to their body weights.

Three test groups received daily treatment with the phenolic extract of *Z. zanthoxyloides* leaves at low, medium and high doses (Aloke *et al.*, 2012) of 100.0, 200.0 and 400.0 mg/kg body weight respectively; whereas the remaining animals served as the untreated diabetic (negative control) group, the reference group which was treated with glibenclamide (0.5 mg/kg body), and the normal control which received no treatment. Extracts and drugs were solubilized in olive oil and the duration of treatment was fourteen (14) days. All administrations were done orally. The glycaemic levels of the rats were measured in five days intervals. The animals were sacrificed on the last day and blood and liver samples were collected for biochemical analyses.

3.2.6 Serum Preparation

The blood of the sacrificed animals was transferred to a plain sample bottle. The blood was allowed to clot by leaving it undisturbed at room temperature for over 30 minutes. The clotted blood was centrifuged at 1,000-2,000 x g for 10 minutes. The serum (supernatant) was thereafter extracted from the pellet using a Pasteur pipette and transferd to another plain sample bottle for further analysis.

3.2.7 Liver Homogenate Preparation

The livers of the sacrificed animals were removed aseptically. They were flash-freezed in foil packets using liquid nitrogen and stored at –80°C. The liver samples were crushed on dry ice and stored in a prechilled 5 ml culture tube. About 100 µg of the liver tissue was added to a new chilled tube and 1 ml phosphate buffered saline with 10 µl protease inhibitors (Sigma Cat. no. P-8340) was added. The solution was chilled using wet ice and homogenized at low speed for over 20 seconds. The samples were transfered into 1.7 ml microcentrifuge tubes and centrifuged at 14,000 x g at 4°C for 15 minutes. The supernatants were obtained and transferred to plain sample bottles.

3.2.8 Determination of Glucose Levels

This was determined using the glucose oxidase method with the use of a glucometer.

3.2.9 Determination of Total Cholesterol

Cholesterol was determined according to the method of Sackets (1925) (Use of Liebermann-Burchard reaction).

Principle: The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

Cholesterol ester + H_2O→ Cholesterol + Fatty acids (cholesterol esterase)

Cholesterol + O_2 → Cholesteren-3-one + H2O2 (cholesterol oxidase)

$2H_2O_2$ + phenol + 4-Aminoantipyrine → quinoneimine + $4H_2O$ (peroxidase)

Procedure

Using fresh distilled H_2O, a new gain calibration was performed in cuvette mode. CHOL was selected in the Run Test screen and a water blank was carried out as instructed**.** The following pipettings were made into a cuvette;

	Reagent Blank 50 (µl)	Standard SI (µl)	Sample (µl)
DdH_2O	5	-	-
Standard	-	5	-
Sample	-	-	5
Reagent	500	500	500

The contents were mixed and incubated for 5 minutes at +37^{o}C. It was inserted into the RX Monza flow cell holder and pressed to read within 60 minutes. Cholesterol was then calculated as shown below;

Conc. of cholesterol in sample= ΔA sample/ΔA standard × conc. of standard

3.2.10 High Density Lipoproteins (HDLs)

High Density Lipoproteins were determined using the Colorimetric method of Camilo, Kássio and Francis (2018).

Principle: Low density Lipoproteins (LDLs and VLDLs) in the sample precipitates with phosphotungstate and magnesium ions. The supernatant contains high density lipoprotein HDL, which was spectrophotometrically measured by the reaction below.

Cholesterol + H_2O →cholesterol + Fatty acid (chol. esterase)

Cholesterol + ½ O_2 + H_2O → cholesterol + H_2O_2 (chol oxidasa)

$2H_2O$ + 4-Aminoanipyrine + DCFS→ Quinonenine + $4H_2O$ (perioxidasa)

Procedures

Precipitation:

To effect precipitation, 0.2ml of the sample and 0.5 ml of the reagent A were pipetted into labeled centrifuge tubes. The contents were mixed thoroughly and were allowed to stand for 10 minutes at room temperature. It was then centrifuged at a minimum of 4000 r.p.m for 10 minutes and the supernatant was carefully collected.

Colorimetry:

Reagent B was brought to room temperature and was pipetted into test tubes and labeled as shown below;

	Blank	Standard	Sample
Distilled water	50µl	-	-
HDL cholesterol standard	-	50µl	-
Sample supernatant	-	-	50µl
Reagent B	1.0ml	1-0ml	1.0ml

It was mixed and the tubes were incubated for 10 minutes at 37^{o}C. The absorbance (A) of the standard and sample at 500nm were measured against the blank. The colour was stable for at least 30 minutes and the HDL cholesterol concentration in the sample was calculated using the general formula.

$A_{Sample}/A_{Standard} \times C_{Sample} \times$ dilution factor $= C_{Sample}$

3.2.11 Low Density Lipoproteins (LDLs)

Low Density Lipoproteins were determined using the Colorimetric method.

Principle: Low density Lipoproteins in the sample precipitates with polyvinyl sulphate. Their concentrations were calculated from difference between the serum total cholesterol and the cholesterol in the supernatant after centrifugation (Assmann, Jab, Kohnert, Nolte & Schriewer, 1984). The cholesterol was spectrophotometrically measured by the reaction below.

Cholesterol esters + H_2O →cholesterol + Fatty acid (chol. esterase)

Cholesterol +half O_2 + H_2O → cholesterol + H_2O_2 (chol. oxidasa)

$2H_2O$ + 4-Aminoanipyrine + DCFS→ Quinonenine + $4H_2O$ (perioxidasa)

Procedure

Precipitation:

In this method, 0.4ml of the sample and 0.2 ml of the reagent A were pipetted into labeled centrifuge tubes. The contents were mixed thoroughly and were allowed to stand for 15 minutes at room temperature. It was then centrifuged at a minimum of 4000 r.p.m for 15 minutes and the supernatant was carefully collected.

Colorimetry:

The reagent (cholesterol kit) was brought to room temperature and was pipette into test tubes and labeled as shown below;

	Blank	Standard	Sample
Distilled water	20µl	-	-
HDL cholesterol standard	-	20µl	-
Sample supernatant	-	-	20µl
Reagent A	1.0ml	1-0ml	1.0ml

It was mixed and the tubes were incubated for 10 minutes at 37ºC. The absorbance (A) of the standard and sample at 500nm were measured against the blank. The colour was stable for at least 30 minutes and the LDL cholesterol concentration in the sample was calculated using the general formula.

LDL Cholesterol = total cholesterol - cholesterol in supernatant

3.2.12 Triacylglycerols (TAGs)

Triacylglycerols were determined using the Spectrophotometric method.

Principle*:* Triacylglycerols in the sample originates by means of the coupled reactions below, a coloured complex that can be measured by spectrophotometry (Vishwanath. and Robert, 1984).

Triglyceride + H_2O → Glycerol + Fatty acids (lipase)

Glycerol +ATP →glycerol-3-P + ADP (glycerol kinase)

Glycerol-3-P + O_2 →Dihydroxyacetone-P + H_2O (G-3-P-oxidase)

2H_2O + 4-Aminoantipyrine + 4-Chlorophenol → Quinoneimine +4 H_2O (peroxidase)

Procedures

The reagent was brought to room temperature and was pipette into labeled test tubes as shown below;

	Blank	Standard	Sample
Triglycerides standard (S)	–	10µL	–
Sample	–	–	10µL
Reagent (A)	1.0mL	1.0mL	1.0mL

The contents were mixed thoroughly and incubated for 15 minutes at room temperature (16-25^{o}C) and the absorbance (A) of the standard and sample were measured at 500 nm against the blank. The colour was stable for at least 2 hours. The triacylglycerols concentration in the sample was afterwards calculated using the general formula;

$A_{sample}/A_{standard} \times C_{standard} = C_{sample}$

3.2.13 Assay of Alanine Aminotransferase (ALT) Activity

Alanine aminotransferase (ALT) catalyzes the transamination of alanine to alpha-ketoglutarate to form glutamate and pyruvic acid, which then reacts with 2, 4-dinitro-phenylhydrazine to form hydrazone derivative of pyruvate. Alanine aminotransferase will be determined as described by Reitman and Frankel (1957) using assay kits.

Principle:

ὰ-oxoglutarate + L-alanine →L-glutamate + pyruvate (GPT)

ALP was measured by monitoring the concentration of pyruvate hydrzone formed with 2,4-dinitrophenylhydrazine.

Procedures

The reagents were brought to room temperature, and were pipetted into labeled test tubes as follows: 0.5ml of reagent 1 was placed into tubes labeled reagent blank and sample. Then 0.1ml of sample was pipetted into sample tubes, while 0.1ml of distilled water was pipetted the reagent blank and thoroughly mixed. The setup was incubated at 37^{o}C for exactly 30 minutes. Then 0.5ml of reagent 2 was added into both the blank and sample tubes, mixed thoroughly and incubated further for exactly twenty (20) minutes at room temperature (20-25^{o}C). Into each of the tubes, 5.0ml of 0.4M NaOH was added into both blank and sample tubes, mixed thoroughly and allowed to stand at room temperature for 5 minutes. The optical densities of the samples were measured against the reagent blank at 546nm in a spectrophotometer. The activity of ALT in the serum was read from the ALT calibration curve in U/L.

3.2.14 Assay of Aspartateaminotransferase (AST) Activity

Aspartate aminotransferase (AST) catalyzes the transamination of aspartate to alpha-etoglutarate to form glutamate and oxaloacetate, which then reacts with 2,4-dinitro-phenylhydrazine to form hydrazone derivative of oxaloacetate, Aspartate aminotransferase will be determined as described by Reitman and Frankel (1957) using assay kits.

Principle:

ὰ-oxoglutarate + L-asparate →L-glutamate + Oxaloacetate (GOT)

AST was measured by monitoring the concentration of oxaloacetate hydrzone formed with 2,4-dinitrophenylhydrazine.

Procedures

The reagents were brought to room temperature, and were pipetted into labeled test tubes as follows: 0.5ml of reagent 1 was placed into tubes labeled reagent blank and sample. Then 0.1ml of sample was pipetted into sample tubes, while 0.1ml of distilled water was pipetted the reagent blank and thoroughly mixed. The setup was incubated at 37^{0}C for exactly 30 minutes. Then 0.5ml of reagent 2 was added into both the blank and sample tubes, mixed thoroughly and incubated further for exactly twenty (20) minutes at room temperature (20-25^{o}C). Into each of the tubes, 5.0ml of 0.4M NaOH was added into both blank and sample tubes, mixed thoroughly and allowed to stand at room temperature for 5 minutes. The optical density of the sample was measured against the reagent blank at 546nm in a spectrophotometer. The activity of AST in the serum was read from the AST calibration curve in U/L.

3.2.15 Assay of Alkaline Phosphatase (ALP) Activity

Serum alkaline phosphatase was determined as described by Rec GSCC (1972). Serum alkaline phosphatase catalyses the hydrolysis of a colourless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which, at alkaline pH values turns into a pink colour that can be determined photometrically at 550 nm.

Principle:

p-nitrophenylphosphate + H_2O→ phosphate + p-nitrophenol

Composition of Reagents

A. Reagent; Diethanolamine 1mol/L, magnesium chloride 0.5mmol/L, pH 9.8

B. Reagent; p-nitrophenylphosphate 10mml/L

Reagent Preparation

The working reagents were prepared by transferring the contents of reagent B via into a reagent A bottle. The reagent are mixed by shaking gently

Procedures

The reagents were brought to room temperature, and reagents were pipetted into labeled cuvettes as follows: 1.0ml of the ALP reagent was pipetted into a cuvette serving as blank and sample cuvette. Into the blank, 0.02ml of distilled water was added and used to zero the spectrophotometer. Into another cuvette 1.0ml of ALP reagent was pipetted and 0.02ml of serum was added, mixed and initial absorbance read. The timer was started simultaneously and the absorbance read after 1minute, 2 and 3minutes interval respectively.

The ALP activity was calculated as using the formular:

ALP activity (U/L) = (ΔA 405nm/min) X 2760

Where; ΔA= Change in absorbance, min = minute.

3.2.16 Determination of Total Proteins

Biuret method of Tietz (1991) was used.

Principle: protein contains a large number of peptide bonds. When treated with Cu^{2+} ions in a moderately alkaline medium, a blue colour chelated complex of unknown composition were formed between the Cu^{2+} ions, carbonyl group and amide group of the peptide bonds. An analogous reaction takes place between the cupric ion and the organic compound. Biuret (NH_2-C-NH_2), hence, the reaction is called Biuret reaction. The intensity of the colour produced is proportional to the number of peptide bonds undergoing the reaction.

Procedure

The reagents were brought to room temperature, and were pipetted into labeled test tubes as

follows: 1.0ml of reagent A was placed into tubes labeled reagent blank, standard and sample. Then 0.02ml of distilled water, standard and sample was pipetted into reagent blank, standard and sample tubes respectively. The set-up was thoroughly mixed and incubated at room temperature (20-25^{o}C) for 30 minutes. The absorbance of the sample and standard was measured against the reagent blank at 546nm in a spectrophotometer. The total protein concentration was then calculated using the formular:

Total protein concentration (g/l) = $\frac{\text{A sample}}{\text{A standard}}$ x c standard (70 g/l)

3.2.17 Determination of Serum Albumin

Estimation of serum total Albumin was done using the method of Doumas *et al.* (1971).

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

Procedure

The reagents were brought to room temperature, and were pipetted into labeled test tubes as follows: 1.0ml of reagent A was placed into tubes labeled reagent blank, standard and sample. Then 0.01ml of distilled water, standard and sample was pipetted into reagent blank, standard and sample tubes respectively. The set-up was thoroughly mixed and incubated at room temperature (20-25^{o}C) for 1 minute. The absorbance of the sample and standard was measured against the reagent blank at 630nm in a spectrophotometer.

The albumin concentration in the sample was then calculated using the formula;

$A_{sample}/A_{standard} \times C_{standard} = C_{sample}$ (g/L)

3.2.18 Determination of Bilirubin Content

This was determined colorimetrically according to the method described by Jendrassic and Grof (1938) using assay kits.

Principle: Conjugated bilirubin reacts with diazotized sulphanilic acid in alkaline medium to form a blue coloured complex. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin, by the reaction with diazotized sulphanilic acid.

Total Bilirubin

Procedure

The reagents were brought to room temperature, and test tubes were labeled as follows: Reagent blank, Sample blank, sample and standard, then 1.0ml of reagent AT was placed into tubes labeled sample blank, and equivalent volume of working reagent was pipetted into the tubes labeled reagent blank, sample and Standard. Furthermore, 0.1ml distilled water was added into the reagent blank, 0.1ml standard to standard tube, while 0.1ml sample was added to the sample blank and samples respectively. The set-up was vortexed and incubated at room temperature (20-25^{o}C) for 30 minutes. The absorbance (A) of samples blanks was read in a spectrophotometer at 578nm against distilled water. The absorbance (A) of samples and standard were read in a spectrophotometer against the reagent blank at 578nm.

Total bilirubin was calculated using the formular;

$$C_{sample} = \frac{A_{sample} - A_{sample\ blank}}{A_{standard}} \times C_{standard}$$

Direct Bilirubin

Procedure

The reagents were brought to room temperature, and test tubes were labeled test tubes as follows: Reagent blank, Sample blank, sample and standard, then 2.0ml of 0.9% NaCl was placed into tubes labeled sample blank, and equivalent volume of working reagent was pipetted into the tubes labeled reagent blank, sample and Standard. The set-up was vortexed and incubated at room temperature (20-25^{o}C) for 10 minutes. The absorbance (A) of samples blanks was read in a spectrophotometer at 546nm against distilled water. The absorbance (A) of samples and standard were read in a spectrophotometer against the reagent blank at 546nm.

Total bilirubin was calculated using the formular;

$$C_{sample} = \frac{A_{sample} - A_{sample\ blank}}{A_{standard}} \times C_{standard}$$

3.2.19 Determination of Ascorbic Acid Content

Ascorbic acid content was determined using the titrimetric method involving the use of 2, 6 dichlorophenol indophenols (Hughes, 1983).

Principle

The blue dye of 2,6 dichlorophenolindophenol is reduced to a colourless leuco compound by ascorbic acid in the solution. Ascorbic acid is a strong reducing agent and thus reduces the

dye 2,6 dichlorophenolindophenol and itself gets converted to dehydro ascorbic acid. After equivalence point is reached, the next drop of dye gives a pink colour to the solution indicating the end point. The dye in this titration is coloured in the oxidized form and colourless in the reduced form.

Procedures

All apparatus including burettes and pipettes were rinsed with 4% metaphosphoric acid prior to the titrations.

Standard titration: In this method, 5 ml of standard ascorbic acid solution was pipetted into a 100 ml conical flask. The burette was filled with the dye solution. The solution was titrated against the dye solution till a light pink color appears which persists for 30 seconds and the dye solution was standardized.

Sample titration: The test solution was taken in a 100 ml volumetric flask and the sample ascorbic acid solution was diluted to the mark with 4% metaphosphoric acid. The contents were mixed well.

Thereafter, 10 ml of this dilute solution was pipette into a 100 ml conical flask and titrated against the dye solution till a light pink colour appears which persists for 30 seconds.

The ascorbic acid content of the sample was calculated afterwards using the following formular;

$$\frac{1 \times b}{a} = c$$

Where a = titre value

b = ml of dye solution reduced by 10ml ascorbic acid

c = mg of ascorbic acid

Since b ml of dye solution is reduced by 10 ml of dilute sample ascorbic acid solution

:. 10 ml of dilute sample ascorbic acid solution contains c mg ascorbic acid.

:. 100 ml of dilute sample ascorbic acid solution contains:

$$\frac{c \times 100}{10} = d$$

:. d = mg ascorbic acid in sample

3.2.20 Determination of Glutathione (GSH) Content

Glutathione (reduced) was measured according to the method of Ellman (1959) as described by Raja *et al* (2007).

Principle

Reduced glutathione (GSH) forms the bulk of non-protein sulfhydryl goups. This method is based on the formation of relatively stable yellow colour when Ellman's reagent is added to a sulfhydryl compound. 2-nitro-5-thiobenzoic acid, the chromophoric product resulting from the reaction of Elmman's reagent with reduced glutathione.

Procedures

Equal quantity of the liver homogenate was mixed with 10% trichloroacetic acid and centrifuged to separate the proteins. To 0.25ml of this supernatant, 2.25 mL of 5, 5-dithio, bis (2-nitrobenzoic acid) in phosphate buffer (pH 8.4) was added. The mixture was vortexed and the absorbance of mixture read at 412 nm within 15 min. Absorbance of glutathione was calculated from the standard calibration curve (y = Mx) prepared by plotting absorbance of standard glutathione concentrations against their standard concentrations when subjected to the same experimental conditions.

3.2.21 Determination of Malondialdehyde (MDA) Content

Malondialdehyde content was estimated colorimetrically using the thiobarbituric acic reactive substance (TBARS) assay kit (Wasowich, Neve and Peretz, 1993).

Principle

MDA forms a 1:2 adduct with thiobarbituric acid. The MDA-TBA adduct formed from the reaction of MDA in samples with TBA can be measured colorimetrically. TBARS levels are determined from a Malondialdehyde equivalence standard.

Procedures

All reagents were prepared and mixed thoroughly before use. Each MDA-containing sample and standard was assayed in duplicate. Initially, 100 µL of the samples and MDA standards were added to separate centrifuge tubes. Then, 100 µL of the SDS Lysis Solution was added to both the samples and the MDA standards and mixed thoroughly. The samples were afterwards incubated for 5 minutes at room temperature. Afterwards, 250 µL of TBA Reagent was added to each sample and standard to be tested. Each tube was closed and incubated at 95°C for 45-60 minutes. The tubes were removed and cooled to room temperature in an ice bath for 5 minutes. All sample tubes were centrifuged at 3000 rpm for 15 minutes and the supernatants were removed from samples for further analysis. Then 200

µL of the MDA standards and samples were transferred to a microplate compatible with a spectrophotometric plate reader and a 0µM blank control was included. It is recommended that duplicates of each standard and sample should be read. The absorbance of the standard and samples were read in duplicates at 532nm. The MDA content in the samples was then determined by comparison with the predetermined MDA standard curve.

3.2.22 Histological Study of Rat Tissues

The pancreas of the animals were removed and fixed in a 10% solution of formaldehyde. The tissues were dehydrated because the reagents used at a later stage were immiscible with water. Varying concentration of isopropyl alcohol, that is, 70%, 80%, 90%, 96%, and 100%, was used for the dehydration. The minimum time for dehydration between two different concentrations was 1h.

The fixed tissues were then cleared in xylene and embedded in paraffin wax. The sections (5µm) from each of the tissues were examined using a light microscope (×40) after staining with hematoxylin and eosin dye.

3.2.23 Statistical Analysis

Data were evaluated using the SPSS/10.00 software. Results were expressed as Mean ± Standard Deviation. Comparison between groups was done using ANOVA. Values were considered statistically significant at 95% confidence level ($P<0.05$).

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Results

4.1.1 Total Phenolics and Flavonoid Contents

The results of the total phenolic and flavonoid contents of the plant extract are presented in table 4.1 below. The result showed that the total phenolic in the plant extract was 7.69 ± 1.45mg tannic acid equivalent/g extract while the total flavonoids was 39.76 ± 1.33mg quercetin equivalent/g extract.

Table 4.1: Total Phenolics and Flavonoid Contents of Samples

Parameters	Values
Total Phenolics (mg Tannic acid equivalent/g extract)	7.69 ± 1.45^{a}
Total Flavonoids (mg Quercetin equivalent/g extract)	39.76 ± 1.33^{b}

*Values are mean ± SD of triplicate determination. Values on the same column bearing different superscript letters are significantly different ($p<0.05$).

4.1.2 Glucose Levels

The results of the glucose levels of animal samples treated within intervals of 5 days for over 15 days are presented in table 4.2 below. The effects of daily doses of the total phenol content of *Zanthoxylum zanthoxyloides* on blood glucose of alloxan-induced rats are presented in table 4.2. There was no significant difference ($p>0.05$) in fasting blood glucose in diabetic control group on day 0 (346.0±14.0mg/dL), day 5 (279.0±23.0 mg/dL), day 10 (349.33±13.5 mg/dL), and day 15 (341.33±5.5 mg/dL). However, on administration of D. 100, no significant difference was found on day 0 (232.0±12.16 mg/dL) and day 5 (202.66±15.30 mg/dL), but there was significant difference ($P<0.05$) on day 10 (159.33±2.86 mg/dL) and day 15 (119.33±8.38 mg/dL). All the rats treated with D. 200 showed no significant difference ($p>0.05$) on day 0 (228.8±13.0 mg/dL) while there was a significant difference on day 5 (144.66±12.89 mg/dL), day 10 (80.33±8.14 mg/dL), and day 15 (97.0±4.35 mg/dL). Administration of D. 400 shows no significant reduction on day 0 (250±21.4 mg/dL) and day 5 (225.8±22.1 mg/dL) while a significant reduction occurred on day 10 (172±15.8 mg/dL) and day 15 (98.2±9.75 mg/dL). There was also significant reduction of blood sugar on day 5 (171.0±2.73 mg/dL), 10 (96.6±8.38 mg/dL) and 15 (86.2±7.01 mg/dL) on administration of the D.Std. It therefore becomes important to mention that there was significant difference ($p>0.05$) in fasting blood glucose between diabetic group treated with the extract and the untreated diabetic group.

Table 4.2: Glucose levels of animal samples

	Day 0 (mg/dL)	Day 5 (mg/dL)	Day 10 (mg/dL)	Day 15 (mg/dL)
Control	68.2 ± 2.68[a]	67.6 ± 6.80[a]	75.6 ± 6.42 [ab]	74.0 ± 6.44 [ab]
Diabetic Control	346.0 ± 14.0 [c]	279.0 ± 23.0 [c]	349 ± 13.1 [c]	341.33 ± 5.6 [c]
Diabetic + 100mg/kg bw Z. Zanthoxyloides	232.0 ± 12.5 [c]	202.66 ± 15.53 [c]	159.33 ± 2.78 [b]	119.33 ± 8.02[ab]
Diabetic + 200mg/kg bw Z. Zanthoxyloides	228.8 ± 13.4 [c]	144.66 ± 12.89 [b]	80.33 ± 8.14 [a]	97.0 ± 4.35 [a]
Diabetic + 400mg/kg bw Z. Zanthoxyloides	250 ± 21.0[c]	225.8 ± 22.3 [c]	172.2 ± 15.8 [b]	98.2 ± 9.6 [a]
Diabetic + 5mg/kg bw Glibenclamide	267.4 ± 26.19 [c]	171.0 ± 2.73 [b]	96.6 ± 8.32 [a]	86.2 ± 7.04[a]

*Values are mean ± SD of triplicate determination. Values on the same column bearing different superscript letters are significantly different ($p<0.05$).

4.1.3 Lipid Profile

Total Cholesterol

The total cholesterol level was significantly increased in diabetic control group (175.79±5.69mg/dL) when compared to the normal control group (91.25±1.34 mg/dL). The cholesterol levels of 100mg/kg b.w, 200mg/kg b.w, and 400mg/kg b.w was significantly reduced to (144.25±2.84 mg/dL), (150.44±4.28 mg/dL), and (128.23±3.8 mg/dL) respectively when compared to the diabetic control group while that of the standard drug reduced to (158.52±3.8 mg/dL).

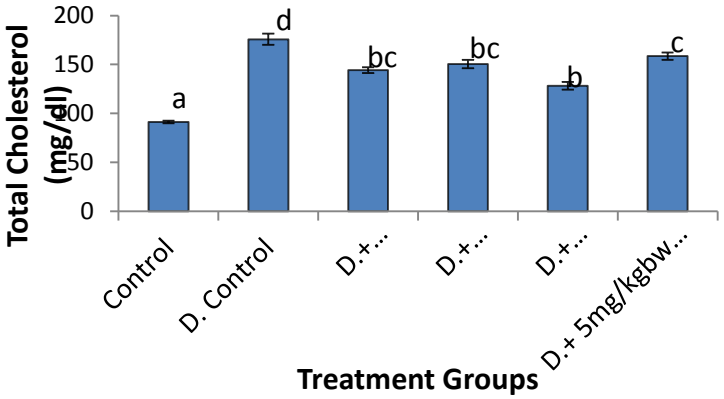


Figure 4.1: Level of total-cholesterol in both control and diabetic experimental rats. Values with different letters are significantly different from each other at $p<0.05$.

High Density Lipoproteins (HDLs)

In this study, there was a significant ($p<0.05$) increase in the experimental groups of 200mg/kg (97.72±1.47 mg/dL), 400mg/kg (100.58±3.71 mg/dL) and glibenclamide (102.92±6.04 mg/dL) when compared with the diabetic control (62.90±1.09 mg/dL).

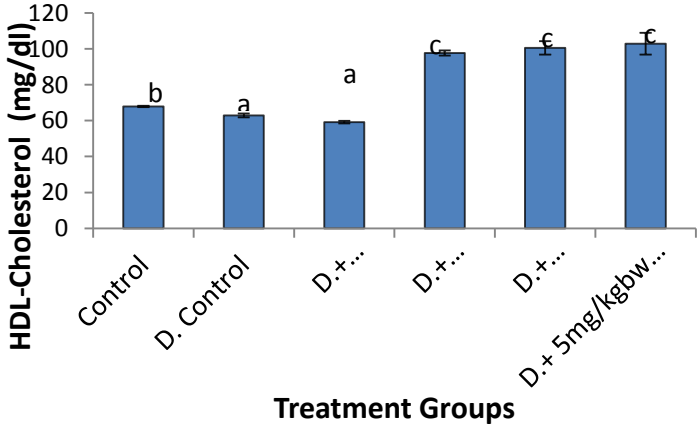


Figure 4.2: Level of HDL-cholesterol in both control and experimental rats. Values with different letters are significantly different from each other at $p<0.05$.

Low Density Lipoproteins (LDLs)

In this study there was a significant ($p<0.05$) decrease in the experimental groups of 100mg/kg (76.04±4.22mg/dL), 200mg/kg (47.28±3.24 mg/dL), 400mg/kg (22.57±3.04 mg/dL) and glibenclamide (54.57±8.81 mg/dL) when compared with the diabetic control (101.82±3.87 mg/dL).

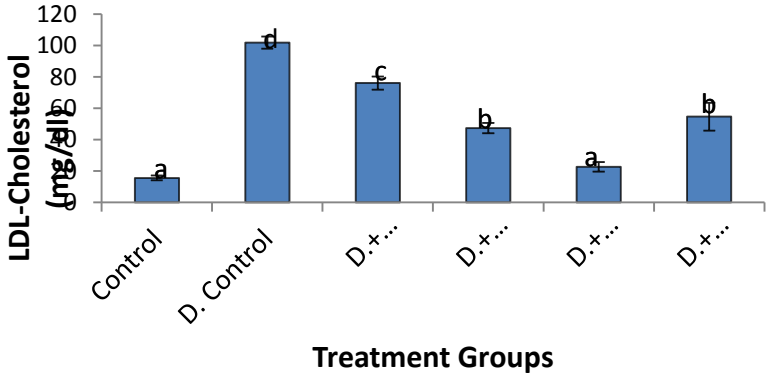


Figure 4.3: Level of LDL-cholesterol in both control and experimental rats. Values with different letters are significantly different from each other at $p<0.05$.

Very Low Density Lipoproteins (VLDLs)

In this study there was a significant ($p<0.05$) decrease in the experimental groups of 100mg/kg (9.03±0.71 mg/dL), 200mg/kg (5.43±0.43 mg/dL), 400mg/kg (5.07±0.53 mg/dL) and glibenclamide (1.02±0.16 mg/dL) when compared with the diabetic control (11.06±0.71 mg/dL).

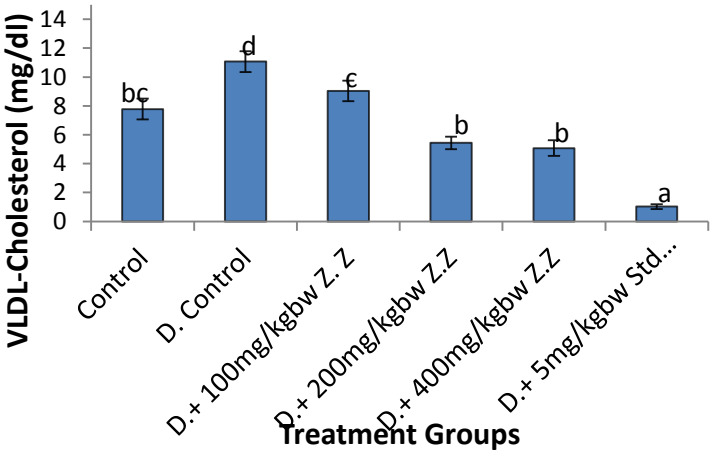


Figure 4.4: Level of VLDL-cholesterol in both control and experimental rats. Values with different letters are significantly different from each other at $p<0.05$.

Low Density Lipoproteins-High Density Lipoproteins Ratio (LDL: HDL)

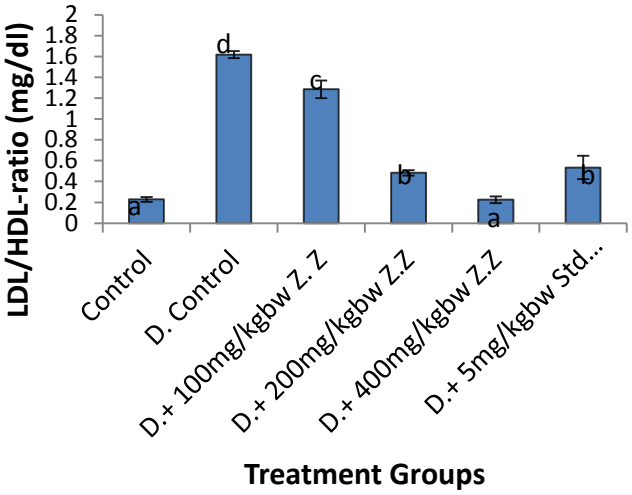


Figure 4.5: Low Density Lipoproteins-High Density Lipoproteins Ratio in both control and experimental rats. Values with different letters are significantly different from each other at $p<0.05$.

Triacylglycerols (TAGs)

TAG was increased in diabetic groups (55.32±3.58 mg/dL) in comparison with the normal control group (38.91±3.56 mg/dL). The TAGs were decreased significantly in the diabetic groups treated with 100mg/kg, 200mg/kg, 400mg/kg and glibenclamide (45.17±3.58 mg/dL, 27.17±2.17 mg/dL, 25.38±2.69 mg/dL and 5.12±0.83 mg/dL respectively) when compared to the diabetic control group.

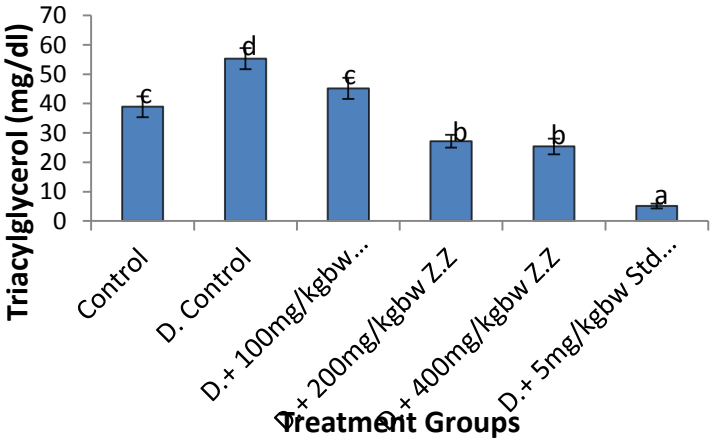


Figure 4.6: Level of triacylglycerol in both control and experimental rats. Values with different letters are significantly different from each other at $p<0.05$.

4.1.4 Liver Function Tests

Alanineaminotransferase (ALT)

Hepatic enzyme, alanine aminotransferase (ALT) activity is shown in Figure 4.7. Serum ALT levels were significantly increased in diabetic rats (78.34±13.04U/L). Treatment of the diabetic with the extract in 100mg/kg (51.90±16.28 U/L), 200mg/kg (23.02±7.56 U/L), 400mg/kg (25.32±3.91 U/L) and glibenclamide (24.82±3.07 U/L) inhibited the increase in serum ALT concentrations in comparison with the diabetic rats that were not treated.

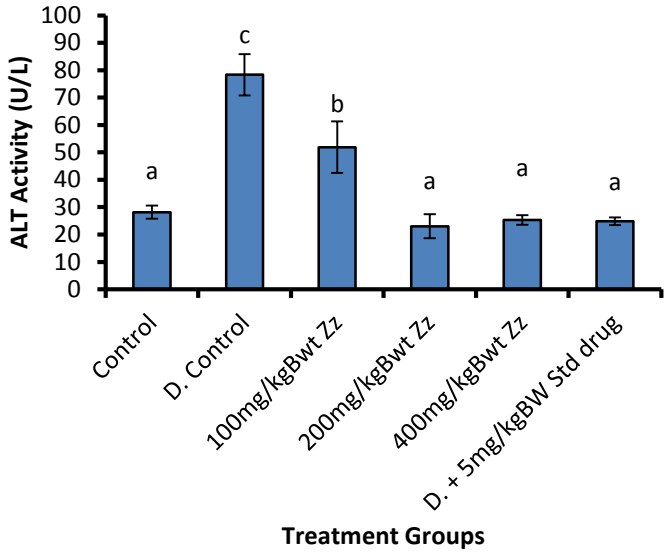


Figure 4.7: Activity of ALT in diabetic rats and non-diabetic rats. Bars represent mean ±S.D of four replicate determinants. Bars with different letters are significantly significant at ($p<0.05$).

Aspartateaminotransferase (AST)

There was a significant decrease (P<0.05) in the activity of AST values as in treated rats as shown in Figure 4.8. Treatment with *Z. zanthoxyloides* phenolic total phenol extract at the dose of 200 mg/kg (51.56±20.97U/L), 400mg/kg (50.75±4.14 U/L) and the standard drug (55.72±8.22 U/L) significantly reduced the activity of the enzymes even lower than the control (61.56±8.67U/L). This showed that the extract reduces the blood sugar, and the enzymes are the good indicator of liver function.

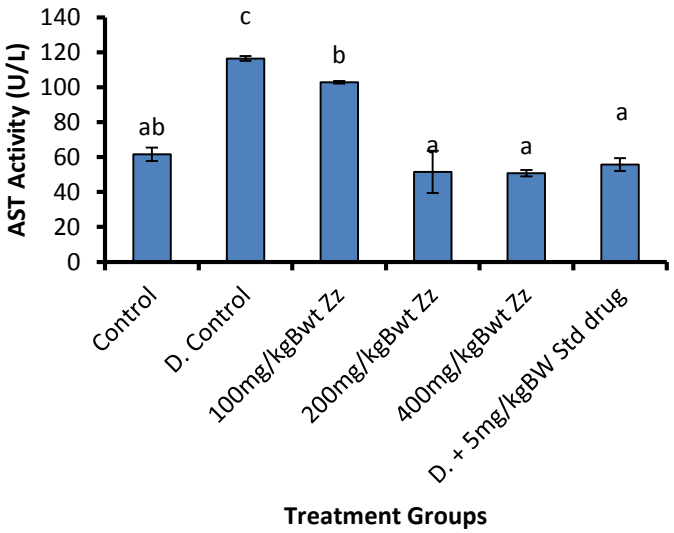


Figure 4.8: Activity of AST in diabetic rats and non-diabetic rats. Bars represent mean ±S.D of four replicate determinants. Bars with different letters are significantly significant at (p<0.05).

Alkalinephosphatase (ALP)

The ALP activity of diabetic group (673.90±147.66 U/L) indicates a significant increase when compared to normal group (266.81±15.38U/L), 200mg/kg (75.44±55.20 U/L), 400mg/kg (149.73±124.24 U/L) and glibenclamide (115.61±31.39 U/L). The group treated with 100mg/kg b/w (612.26±352.82 U/L) reduced the ALP concentration of the rats though not as much as other treated groups. This could be attributed to the concentration factor.

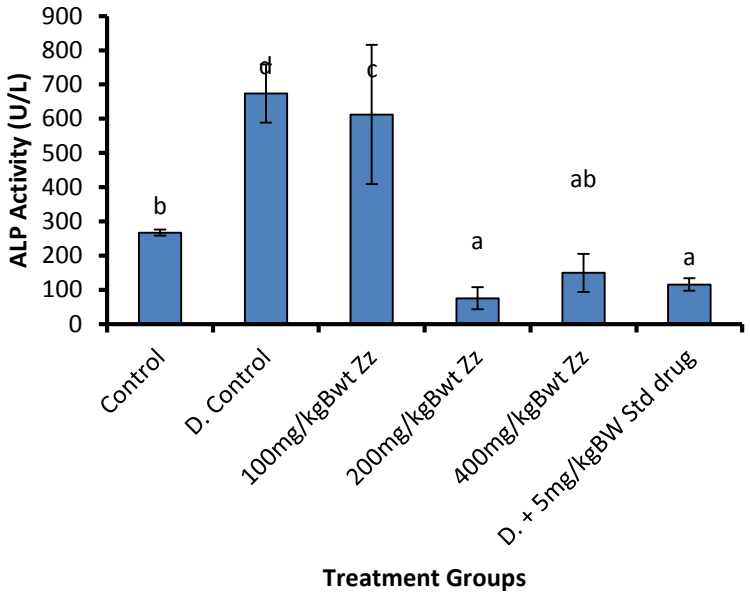


Figure 4.9: Activity of ALP in diabetic rats and non-diabetic Rats. Bars represent mean ±S.D of four replicate determinants. Bars with different letters are significantly significant at ($p<0.05$).

Total Protein

The present studies showed a significant ($p<0.05$) decrease in the levels of total protein of the diabetic rat (50.08±7.60g/dL) when compared to the control (71.47±4.03 g/dL), the 200mg/kg (58.16±18.47 g/dL), 400mg/kg (79.52±7.20 g/dL), and standard drug (58.98±0.84 g/dL) treated samples. The samples that were treated with 100mg/kg b/w (51.64±4.6) of the extract showed a slight though not significant increase in total protein levels when compared to the diabetic untreated rats which could be attributed to the low extract concentration. These results thus, suggest the enhancement of liver functions by the extracts.

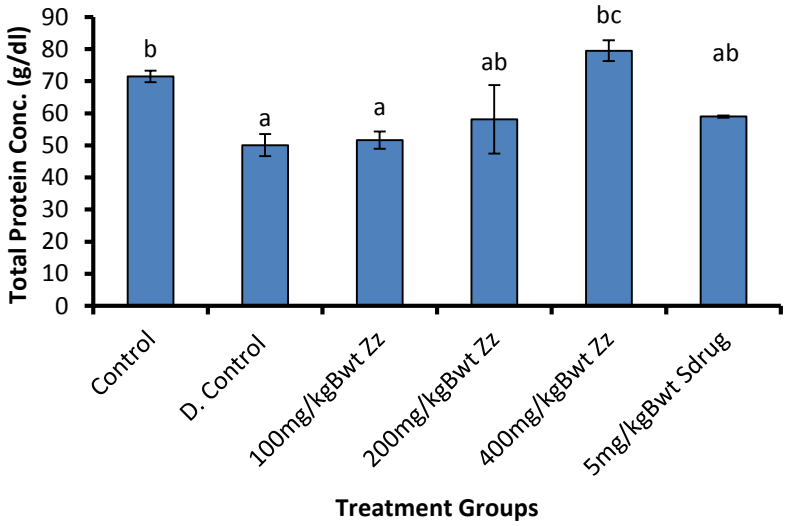


Figure 4.10: Level of total protein in both control and experimental rats. Values with different letters are significantly different from each other at $p<0.05$.

Albumin

Oral administration of the extract restored the albumin in 200mg/kg (29.49±0.27 g/dL), 400mg/kg (32.78±1.37 g/dL), and glibenclamide (34.81±4.78 g/dL) when compared to the diabetic untreated rats (26.95±1.30 g/dL). The samples that were treated with 100mg/kg b/w (27.75±0.50) of the extract showed a slight though not significant increase in total protein levels when compared to the diabetic untreated rats which could be attributed to the low extract concentration.

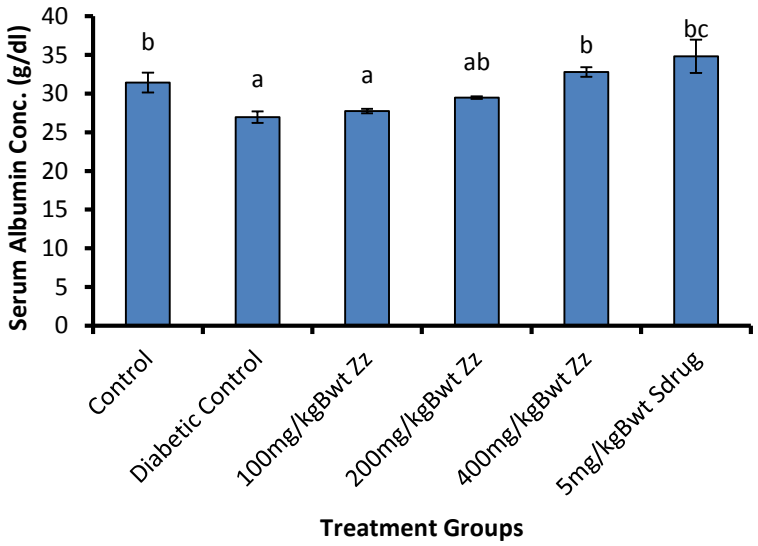


Figure 4.11: Level of serum albumin in both control and experimental rats. Values with different letters are significantly different from each other at $p<0.05$.

Globulin

Globulin decreased significantly in diabetic rats (23.48±12.93g/dL) when compared with the normal control (40.05±2.65 g/dL). It increased significantly ($p < 0.05$) in the experimental group of 200mg/kg (28.67±9.91 g/dL) and 400mg/kg (46.74±6. g/dL), but remained insignificantly different in 100mg/kg (23.89±5.19 g/dL) and glibenclamide (24.17±4.60 g/dL).

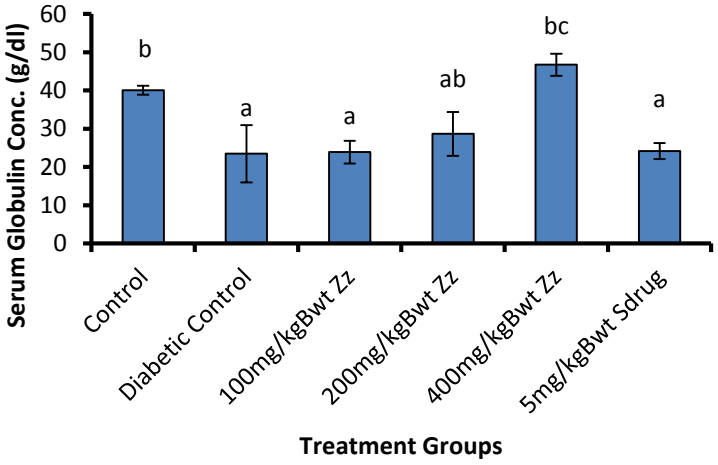


Figure 4.12: Level of serum globulin in both control and experimental rats. Values with different letters are significantly different from each other at $p<0.05$.

Total Bilirubin

In this study, the reduction of bilirubin in some of the treated rats 400mg/kg (1.03±0.38mg/dL), and glibenclamide (1.15±0.27 mg/dL) were not much when compared it to the control (1.35±0.1 mg/dL). But treatment with 100mg/kg (0.46±0.18 mg/dL) and 200mg/kg (0.49±0.41 mg/dL) of the extract were able to reverse the condition in diabetic rats by lowering the level of total bilirubin very significantly. The decrease in the administration of this concentration, suggested that it offered protection.

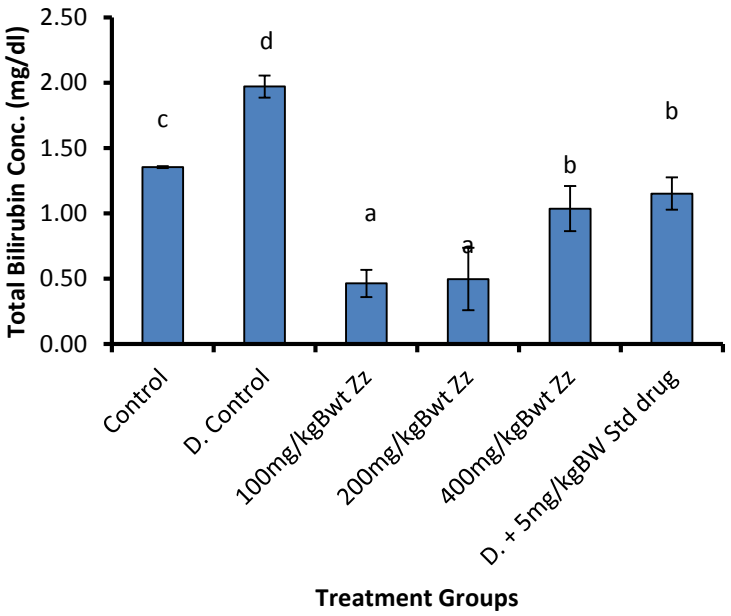


Figure 4.13: Level of total bilirubin in both control and experimental rats. Values with different letters are significantly different from each other at $p<0.05$.

Direct Bilirubin

In this study, the reduction of direct bilirubin in the treated rats was very significant compared to the diabetic untreated group (1.0±0.13g/dL). The treated groups had; 100mg/kg (0.61±0.19 g/dL), 200mg/kg (0.17±0.05 g/dL), 400mg/kg (0.38±0.23 g/dL), and standard drug (0.50±0.33 g/dL) while the normal control group had a direct bilirubin level of (0.33±0.09 g/dL). The decreased in the administration of this concentration, suggested that it offered protection.

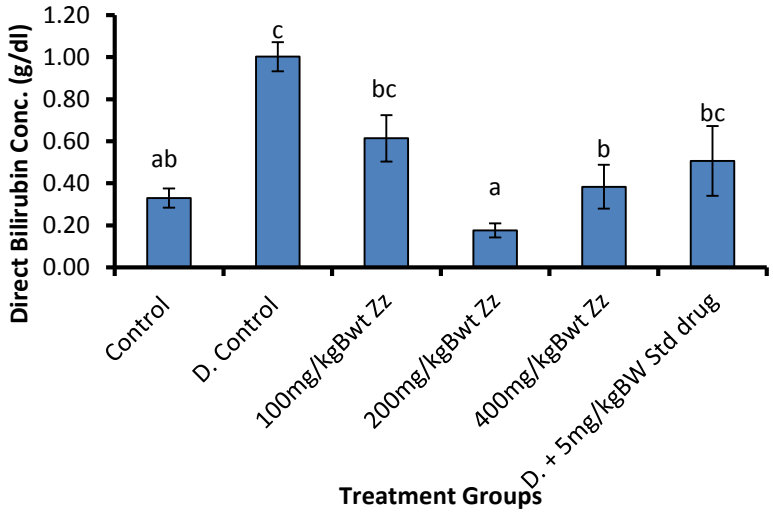


Figure 4.14: Level of direct bilirubin in both control and experimental rats. Values with different letters are significantly different from each other at $p<0.05$.

4.1.5 Oxidative Stress Parameters

Vitamin C (Ascorbic Acid)

Serum vitamin C content was generally significantly (P≤0.05) increased when *Z. zanthoxyloides* phenolic contents of 100mg/kg (249.78±0.84mg/dL), 400mg/kg (320.33±130.27 mg/dL) and glibenclamide (253.67±19.90 mg/dL) were orally administered to the rats when compared to diabetic control rats (204.00±23.83 mg/dL). Vitamin C can act as a co-antioxidant by regenerating α-tocopherol radicals produced during scavenging of reactive oxygen molecules (Pecker, 1997). The high serum content of vitamin C recorded in this study, therefore, is possibly contributed from the leafy vegetables.

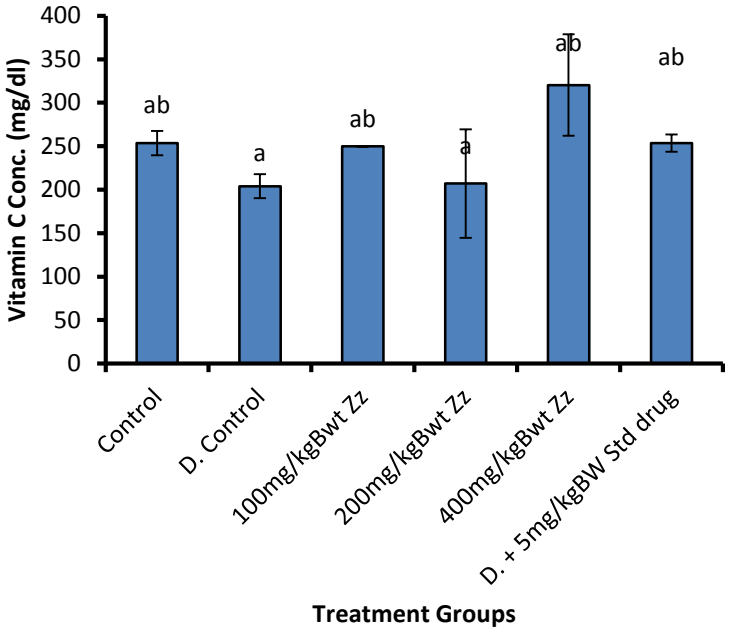


Figure 4.15: Level of Vitamin C in both control and experimental rats. Values with different letters are significantly different from each other at p<0.05.

Glutathione (GSH)

GSH reduced in diabetic groups (14.09±1.45) in comparison to its increase in the treated diabetic rats 100mg/kg (20.07±0.22mg/dL), 200mg/kg (23.87±4.17mg/dL), 400mg/kg (23.33±1.95 mg/dL) and glibenclamide (27.08±4.40 mg/dL) with *Z. zanthoxyloides*, suggesting that *Z. zanthoxyloides* has the ability to scavenge and neutralize Alloxan induced oxidative stress. This may be related to the presence of terpenoids in the extract.

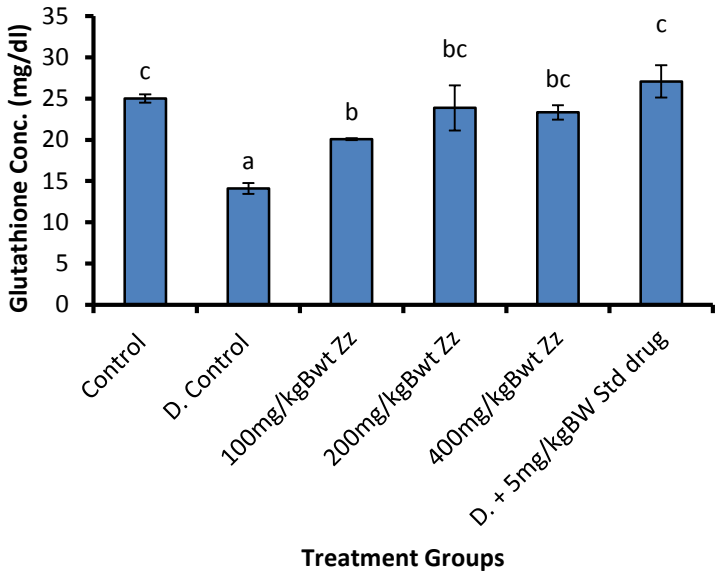


Figure 4.16: Level of Glutathione in both control and experimental rats. Values with different letters are significantly different from each other at $p<0.05$.

Malondialdehyde (MDA)

The generation of free radicals may lead to lipid per oxidation and the formation of several types of damage in diabetes mellitus. The results of this study revealed a significant difference between the diabetic rats (8.25±1.72nmol/L) and the treated rats as well as the control group (3.61±0.78 nmol/L). The MDA levels of the treated rats however were; 100mg/Kg (3.99±0.5 nmol/L), 200mg/Kg (24.93±1.84 nmol/L), 400mg/Kg (7.25±0.72 nmol/L) and standard drug (6.91±1.54 nmol/L).

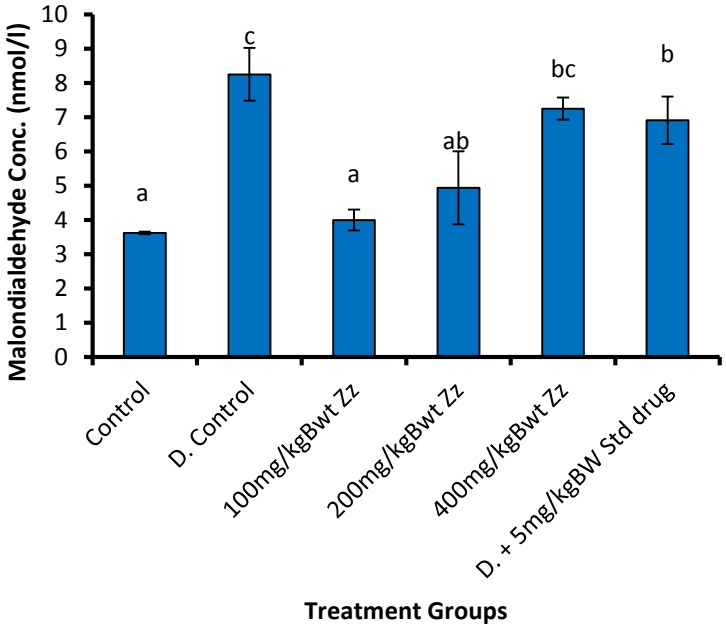


Figure 4.17: Level of malondiadehyde in both control and experimental rats. Values with different letters are significantly different from each other at $p<0.05$.

4.1.6 Histopathology

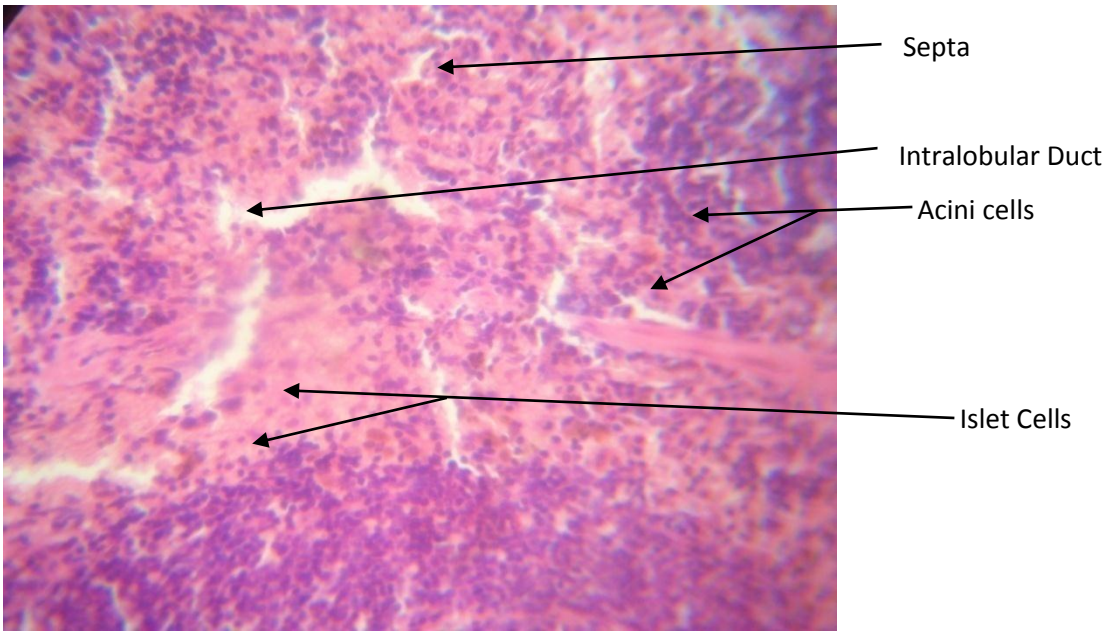


Plate 1: Transverse section of pancreas (control) H & E stain x 400

Plate 1 shows a section typical of the pancreas. Septa extend from the capsule into the gland and divide it into lobules. There is presence of intralobular and interlobular ducts. The gland is made of serous acini. The cells of the acini are highly basophilic (bluish staining); some are pale staining (centroacinar cells). In this normal photomicrograph, at some areas the acini are separated by aggregations cells that form the pancreatic islets (pale staining cells arranged as a group).

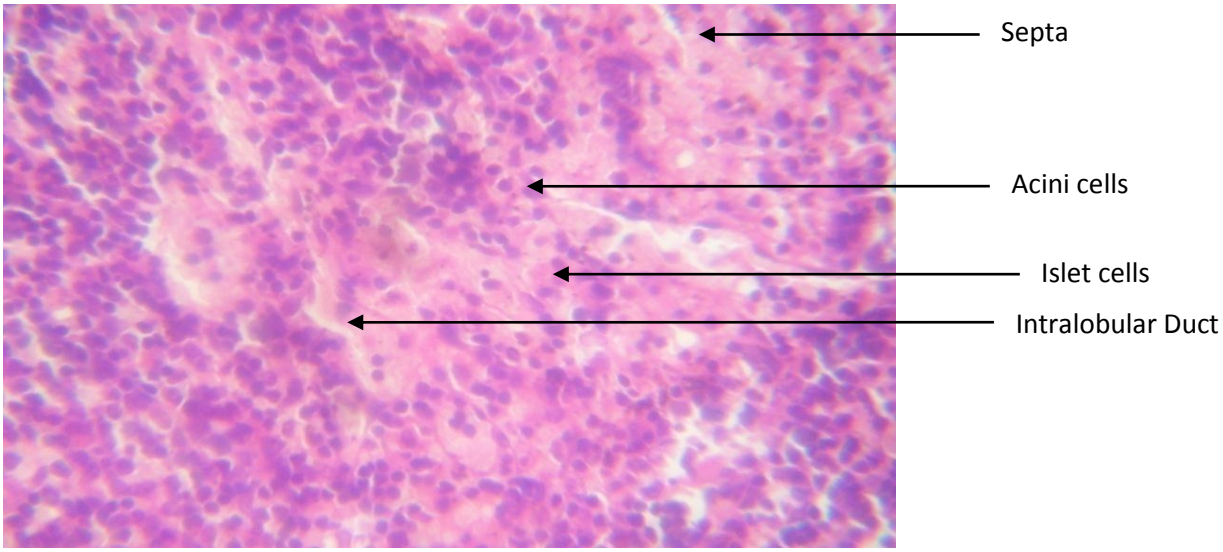


Plate 2: Transverse section of pancreas (diabetic control) H & E stain x 400

Plate 2 shows a section of pancreas that was exposed to diabetic agent. There is distortion of both the acini cells and islets cells. The distortion affected most of the islets cells and many have undergone necrosis. There is scanty of islets cells. The acini cells are numerous and stained basophyilic. Lobulation is more prominent.

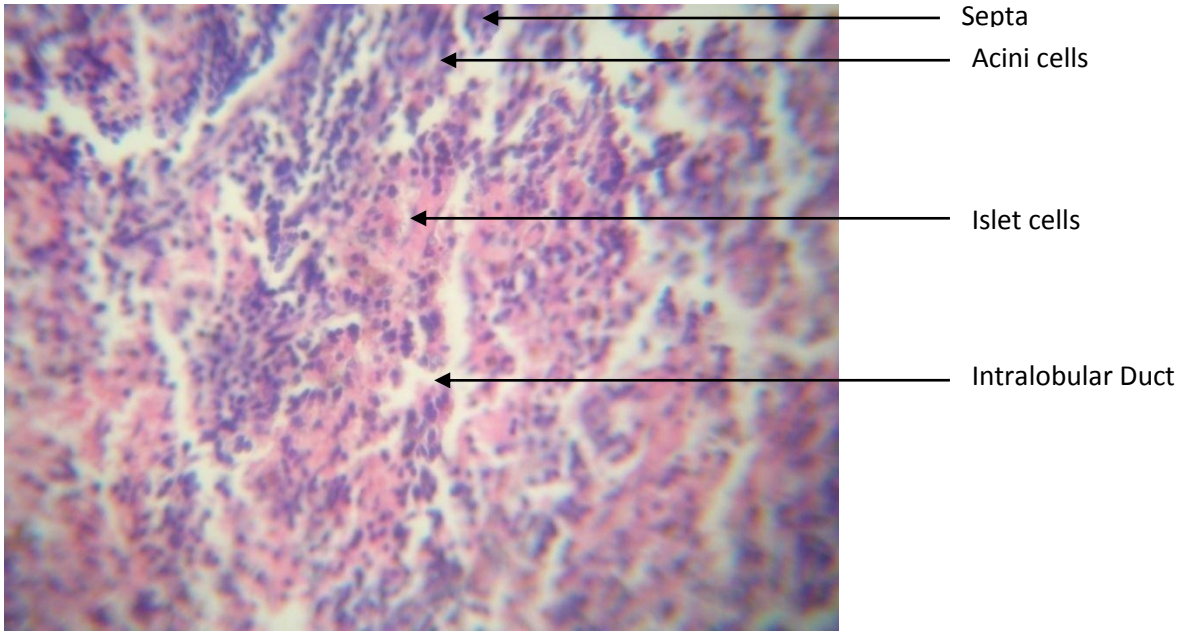


Plate 3: Transverse section of pancreas (diabetic with 100mg/kg bw Z.z.) H & E stain x 400

Plate 3 shows a section of pancreas that received 100mg of the extract. Regeneration of islet cells was very minimal and necrosis was more evident. The acini cells are appeared basophyilic while the few islets cell appeared pale.

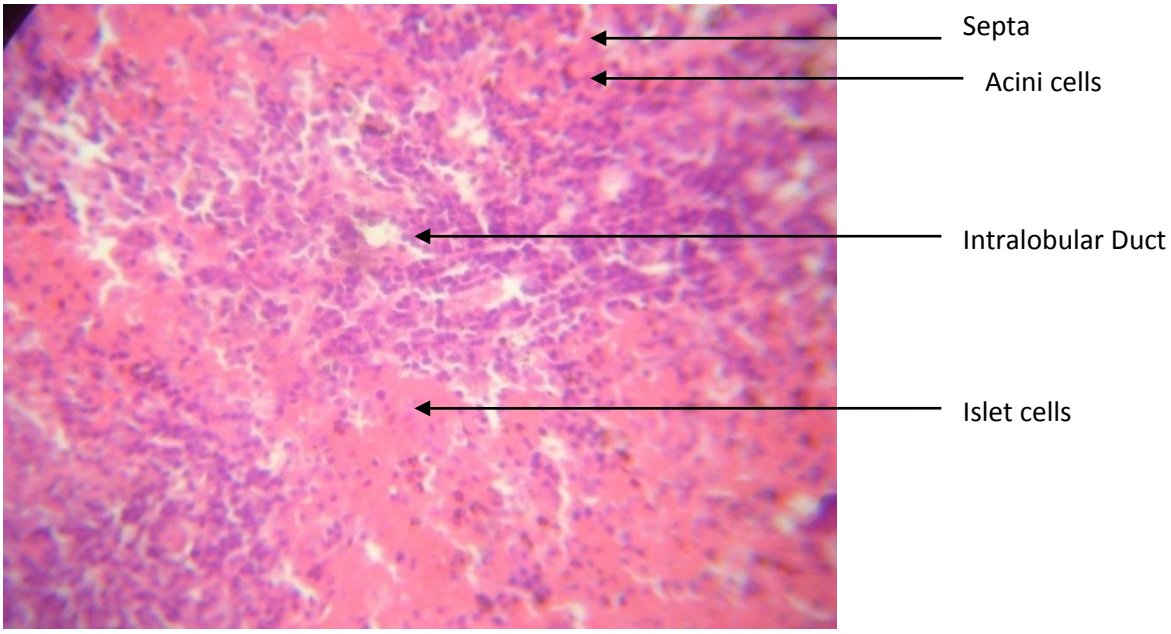


Plate 4: Transverse section of pancreas (diabetic with 200mg/kg b/w Z.z.) H & E stain x 400

Plate 4 shows a section of pancreas that received 200mg of extract. There was a slight increase in the regeneration of islet cells when compared to rats that received 100g of the extract. The acini cells are appeared basophyilic.

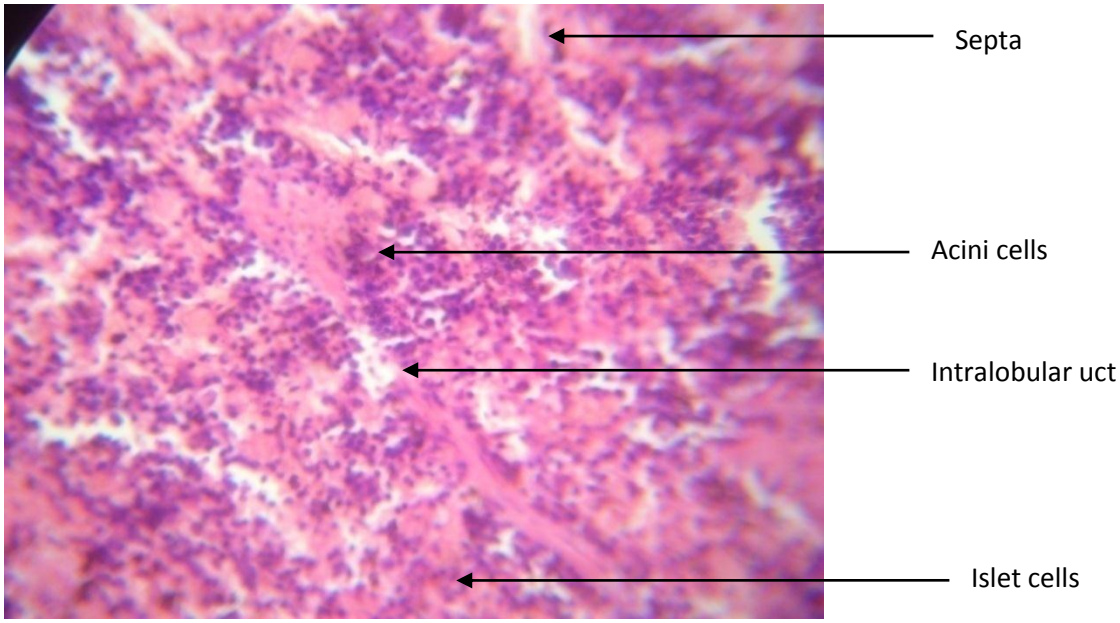


Plate 5: Transverse section of pancreas (diabetic with 400mg/kg b/wZ.z.) H & E stain x 400

Plate 5 shows a section of pancreas that received 400mg of the extract. There was more increase in islet cells regeneration when compared to photomicrograph of rats that received 100g and 200mg of the extracts. The acini cells appeared basophyilic while islets cells appeared pale.

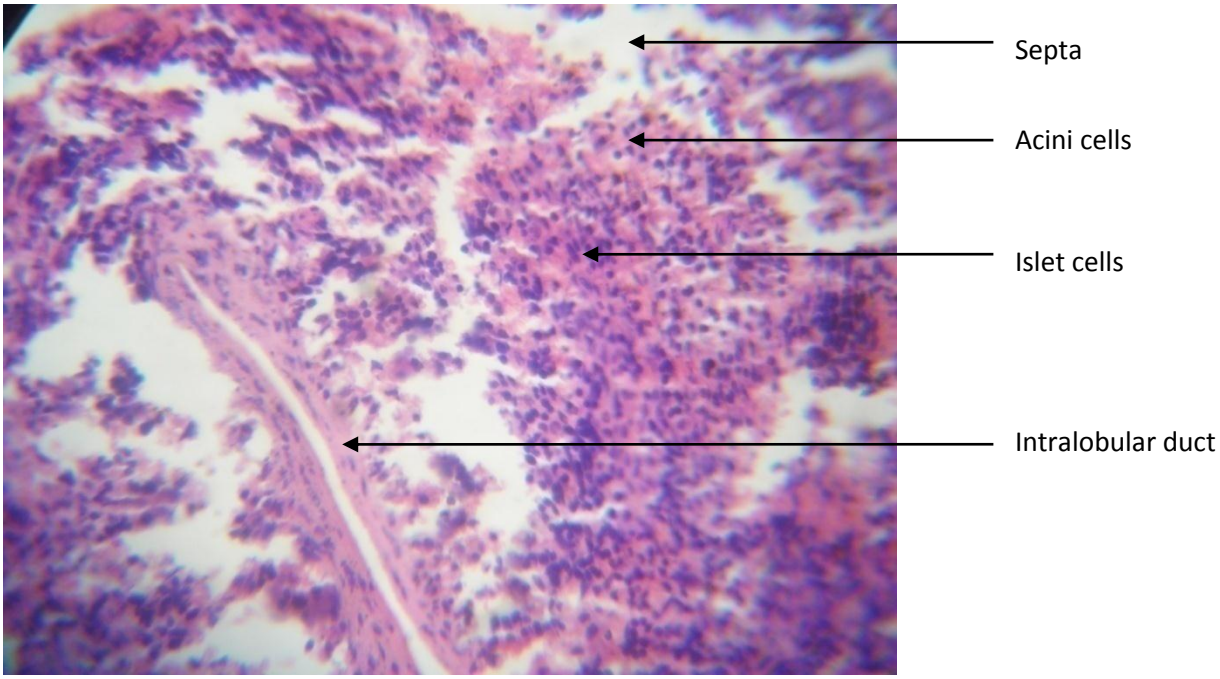


Plate 6: Transverse section of pancreas (diabetic with 5mg/kg b/w S.D.) H & E stain x 400

Plate 6 shows a section of pancreas that received standard drug treatment. The acini cells appeared to be basophyilic and numerous. Areas assumed to be occupied by islets cells appeared empty. Thus was necrosis of the islet cell and regeneration was not evident.

4.2 Discussions

Diabetes mellitus causes disturbances in the uptake of glucose by cells as well as glucose metabolism. Thus, alloxan-induced hyperglycaemia is a very useful experimental way of studying and demonstrating the activity of new hypoglycaemic agents (Srinivasan and Ramarao, 2007). Oral glucose tolerance tests were used to analyze blood glucose levels taken at different regular intervals after repeated treatments with *Z. zanthoxyloides* total phenol extract. Results of the oral glucose tolerance test, using total phenol extract of *Z. zanthoxyloides* (400 mg/Kg BW), indicate significant decrease in blood glucose levels of the alloxan-induced diabetic rats. This suggests that the total phenol extracts of *Z. zanthoxyloides* leaves enhance glucose utilization and hence improve glucose tolerance in diabetic rats.

In addition to antidiabetic effect, *Z. zanthoxyloides* leaves showed improvements in serum lipids in the diabetic rats treated with total phenol extract of leaves of *Z. zanthoxyloides* in comparison to the diabetic control rats. Interesting to note is the fact that the untreated diabetic rats showed significant elevation in HDL-cholesterol, suggesting that the plant has protective effect on the heart. This actually supported a study carried out by Aloke *et al.* (2012), where the rat samples were treated by orally ingesting the *Z. zanthoxyloides* leaves. It is well known that in uncontrolled diabetes, the resultant increases in LDL, triglyceride and total cholesterol were associated with increased morbidity and mortality from coronary artery disease (Arvind, Pradeepa, Deapa & Mohan, 2002). The protective effect of *Z. zanthoxyloides* was seen to be extended to the liver in this study, as shown by increases in total protein and albumin, thus supporting the regenerating capacity of this plant leaves on the pancreatic tissues which are manifested by increased utilization of glucose and protein synthesis.

The activities of liver enzymes like ALT, AST, and ALP was also observed to be reduced in diabetic rats treated with total phenol extract of *Z. zanthoxyloides* leaves when compared to the untreated diabetic group. This shows a similar result with Ebong, Atangwho, Eyong & Egbung (2008) who worked with a different plant extract. The activity of these enzymes is a bio-marker for possible liver injury. Thus, the total phenol content of *Z. zanthoxyloides* leaves has been shown to protect the liver from injury.

The phytochemical analysis of the total phenol extracts of *Z. zanthoxyloides* leaves reported the presence of phenols and flavonoids. Presence of these compounds has been suggested by several authors to be responsible for antidiabetic activity (Alagammal, Agnel & Mohan, 2012). Flavonoids have also been known to regenerate the damaged beta cells in alloxan-induced diabetic rats and act as insulin secretagogues (Mao-Ying, Kavelaars & Krukowski,

2014). Thus, the hypoglycaemic activity of total phenol extracts of *Z. zanthoxyloides* leaves may be due to the presence of hypoglycaemic flavonoids and phenolic acids; however, this also requires further investigation. The antihyperglycaemic effect of *Z. zanthoxyloides* may be attributed to the potentiation of insulin from existing β-cells of the islets of Langerhans. The blood glucose lowering effect of total phenol extract of *Z. zanthoxyloides* leaves was compared with that of glibenclamide, a standard drug which has been in use for many years for treatment of diabetes and acts by stimulating insulin secretion from pancreatic β-cells (Luzi and Pozza, 1997).

The induction of diabetes using alloxan resulted in severe damage of β-cells of the islets of Langerhans (Plate 2). However, after repeated treatment with *Z. zanthoxyloides* (400 mg/Kg BW), for 15 days, there was regeneration of the central β-cells (Plate 5). There was also a notable increase in the number of secretive β-cells which are epithelial cells with ability to regenerate. The *Z. zanthoxyloides* total phenol extract appeared to stimulate the regeneration of β-cells of the islets of Langerhans. Albeit the still low number of the β-cells, the animals were able to maintain glucose levels close to the normal. This therefore also implies that the total phenol extract of *Z. zanthoxyloides* leaves increase the sensitivity of the insulin receptors to insulin.

Previous reports indicate that medicinal plants that possess hypoglycaemic activity act through various mechanisms including improvement in the sensitivity of target cells to the effects of insulin, augmenting glucose-dependent insulin secretion, and stimulating the regeneration of β-cells of islets of Langerhans in pancreas of alloxan-induced diabetic rats (Ayodhya, Kusum & Anjali, 2010). Some of the medicinal plants seem to regulate enzymes of glycolysis, gluconeogenesis, and other pathways (Arya *et al.*, 2012). Active phytochemical compounds act through a variety of mechanisms; however, in this study, identification of the mechanism of action of the extract was not done; thus the suggestions made are only hypothetical.

CHAPTER FIVE
CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

Diabetes is considered to be a metabolic disorder that mainly occurs due to defects in either insulin secretion, insulin action, or both. Diabetes is a disease that can lead to serious problems affecting human health. In the long term, effects can cause micro and macro vascular problems (Mohana, Sandhya & Kiran, 2012). In addition, uncontrolled diabetes can cause many chronic complications, including blindness, heart disease, and renal failure (Mamun-or-Rashid *et al.*, 2014). A significant change occurs in the structure and metabolism of lipid in diabetes. Lipid peroxidation is associated with hyperlipidemia. The liver plays a critical role in glucose, lipid homeostasis, and has an important effect on diabetes. The liver and kidneys participate in the absorption, oxidation, and metabolism of free fatty acids and synthesize cholesterol, phospholipids, and triglycerides. Despite the presence of anti-diabetic drugs in the pharmaceutical market, the treatment of diabetes with medicinal plants is often successful. Herbal medicines and plant components with insignificant toxicity and no side effects are notable therapeutic options for the treatment of this disease around the world (Gupta and De, 2012). Most tests have demonstrated the benefits of medicinal plants containing hypoglycaemic properties in diabetes management. The most common herbal active ingredients used in treating diabetes are flavonoids, tannins, phenolic, and alkaloids (Mamun-or-Rashid *et al.*, 2014). The existence of these compounds implies the importance of the anti-diabetic properties of these plants (Gupta and De, 2012).

The present work therefore has detected the antidiabetic effects of total phenol extracts of *Zanthoxylum zanthoxyloides* leaves in alloxan-induced diabetic rats.

5.2 Recommendations

Plants are natural antioxidants and effective herbal medicines, in part due to their anti-diabetic compounds, such as flavonoids, tannins, phenolic, and alkaloids that improve the performance of pancreatic tissues by increasing the insulin secretion or decreasing the intestinal absorption of glucose. More researches are needed in order to separate the active components of *Zanthoxylum zanthoxyloides* leaf extracts and molecular interactions of their compounds for analysis of their curative properties.

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APPENDICES

INITIAL BODY WEIGHT, BLOOD GLUCOSE LEVELS, AND FINAL ORGAN WEIGHT OF EXPERIMENTAL ANIMALS

GROUP		WEIGHT	BLOOD GLUCOSE (ml/dl)				ORGAN WEIGHT (g)		
			0	5	10	15	Livers	Pancreas	Kidneys
Control	H	130	66	74	84	73	6.2	1.2	1.3
	N	129	72	74	70	72	6.1	1.2	1.3
	B	133	67	60	69	68	6.9	1.4	1.6
	T	134	70	69	80	72	6.1	1.2	1.3
	P	117	66	61	75	85	5.2	0.9	1.0
Diabetic Control	H	183	332	256	436	166	8.3	1.7	1.4
	N	177	360	303	350	335	7.7	1.3	1.3
	B	150	-	-	-	-	-	-	-
	T	178	347	277	361	343	7.1	1.5	1.2
	P	195	-	-	-	-	-	-	-
Diabetic + 100mg/	H	158	238	198	155	111	7.3	1.3	0.83
bw *Z. zanthoxyloides*	N	150	-	-	-	-	-	-	-
	B	150	218	190	161	120	7.25	1.3	0.9
	T	148	-	-	-	-	-	-	-
	P	144	241	220	162	127	7.2	1.3	0.75
Diabetic + 200mg/	H	140	217	141	71	95	8.01	1.69	1.33
bw *Z. zanthoxyloides*	N	141	243	159	84	102	8.5	1.65	1.41
	B	142	-	-	-	-	-	-	-
	T	140	224	134	86	94	9.08	1.60	1.49
	P	140	-	-	-	-	-	-	-
Diabetic + 400mg/	H	165	243	203	153	86	8.42	0.92	1.30
bw *Z. zanthoxyloides*	N	153	237	224	162	90	8.75	1.4	1.60
	B	128	226	184	234	107	8.53	1.16	1.67
	T	160	274	297	234	102	6.75	0.85	1.65
	P	158	270	248	194	106	11.33	2.70	1.81
Diabetic + 5mg/bw	H	173	287	170	90	89	10.5	1.15	2.01
standard drug	N	155	281	173	93	95	9.87	1.20	1.75
	B	165	278	174	107	89	9.39	1.15	1.60
	T	120	222	171	89	78	8.25	1.40	1.55
	P	160	269	167	104	80	11.34	1.11	1.87

PREPARATION OF TREATMENT FOR ANIMAL SAMPLES

For 100mg/kg of *Z. zanthoxyloides*

100g - 100mg

Head - 158g

1000g - 100mg

158 - Xmg

$$Xmg = \frac{158 \times 100}{1000}$$

$$= 158 \times 0.1$$

= 15.8mg - 1ml

Neck = 150 x 0.1
= 15. 0 mg - 1ml

Body = 150 x 0.1
= 15. 0 mg - 1ml

Tail = 148 x 0.1
= 14.8mg1ml

Plain = 144 x 0.1
= 14. 4mg - 1ml

For one day = 15.8 x 15.0 + 15.0 + 14.8 + 14.4
= 75mg - 5ml

For 14 days = 75 x 14 – 5 x 14
= 1050 mg - 70 ml

Additional = 1050 mg - 70ml
Xmg - 80ml

$$Xmg = \frac{80 \times 1050}{70}$$

= 1200mg - 80ml ≈ 1.2g/80ml

For 200mg/kg of *Z. zanthoxyloides*

Head – 140g

1000g - 200mg

140 – Xmg

Xmg = $\frac{140 \times 200}{1000}$

= 28mg – 1ml

Neck = 140.5g x 0.2
= 28.1mg - 1ml

Body = 142g x 0.2
= 28.4.mg - 1ml

Tail = 140 x 0.2
= 28mg - 1ml

Plain = 140 x 0.2
= 28mg x 0.2

For one day = 28 + 28.1 + 28.4 + 28 + 28
= 140.5mg - 5ml

For 14 days = 140.5 x 14 – 5 x 14
= 1967mg - 70ml

Additional = 1967mg - 70ml
Xmg - 80ml
Xmg = $\frac{80 \times 1967}{70}$

= 2248mg - 80ml ≈ 2.24g/80ml

For 400mg/kg of *Z. zanthoxyloides*

100g - 400mg

Head - 165g

1000g - 400mg

165 - Xmg

Xmg = $\frac{165 \times 400}{100}$

= 66mg - 1ml

Neck = 153 x 0.4
= 61.2 mg - 1ml

Body = 128 x 0.4
= 51. 2 mg - 1ml

Tail = 160 x 0.4
= 64mg/ml

Plain = 158 x 0.4
= 63.2mg - 1ml

For one day = 66 + 61.2 + 51.2mg + 64mg + 63.2mg
= 305.6mg - 5ml

For 14 days = 305.6mg x 14 – 5 x 14
= 4278.4mg - 70ml

Additional = 4278.4mg - 70ml
Xmg - 80ml
Xmg = $\frac{80 \times 4278.4}{70}$

= 4889.6mg - 80ml ≈ 4.9g/80ml

Standard Drug (Glibendamide)

If 10mg – 60kg (Adult weight)

Then $\frac{10}{60}$ = 1kg
= 0.17mg/kg

If 1kg - 0.17mg

Then 1000g - 0.17mg

Head – 173g

= $\frac{0.17}{1000 \times 173}$

= 0.00017 x 173

= 0.02941mg

Neck = 0.00017 x 155mg
= 0.026mg - 1ml

Body = 0.00017 x 165mg
= 0. 028 mg - 1ml

Tail = 0.00017 x 120
= 0.0204mg - 1ml

Plain = 0.00017 x 160

= 0.0272mg -1ml

For one day = 0.02941 + 0.026 + 0.028 + 0.0204 + 0.0272
= 0.1310mg - 5ml

For 14 days = 0.1310mg x 14 – 5ml x 14
= 1.83mg - 70ml

Additional = 1.83mg - 70ml
Xmg - 80ml
$Xmg = \frac{80 \times 1.83}{70}$

= 2.09mg ≈ 0.00209g/80ml

Since 2.09mg is small, then we calculate for 5mg which is 1 tablet as shown below:

If 2.09mg - 80ml

Then 5mg – Xml

$Xml - \frac{5 \times 80}{2.09}$

= 191.38ml
;. Solute/solvent ratio will be 5mg/191.38ml

INDUCTION OF DIABETES ON ANIMAL SAMPLES USING ALLOXAN

For 130mg/kg of Alloxan

If 130mg - 1kg

Then for Diabetic Control

Head – 183g

Xmg - 183g

Xmg = $\frac{130}{1000}$ x 183g
= 0.13 x 183g
= 23.79mg

Neck = 176.5
Xmg - 176.5g
Xmg = 0.13 x 176.5g
= 22.95mg

Body = 150
Xmg - 150g
Xmg = 0.13 x 150g
= 19.5mg

Tail = 178g
Xmg - 178g
Xmg = 0.13 x 178g
= 23.14mg

Plain = 195g x 0.1
Xmg - 195
Xmg = 0.13 x 195
= 25.35mg

Total = 23.79 + 22.95 + 19.5 + 23.14 + 25.35
= **114.73mg**

For Diabetic + 100mg/kg B/w *Z. zanthoxyloides*

Head – 158g

Xmg - 158g

Xmg = 0.13 x 158
- 20.54mg

Neck = 150g
Xmg - 150

Xmg = 0.13 x 150g
= 19.5mg

Body = 150g
Xmg - 150
Xmg = 0.13 x 150g
= 19.5mg

Tail = 148g
Xmg - 148g
Xmg = 0.13 x 148g
= 19.24mg

Plain = 144g
Xmg - 144g
Xmg = 0.13 x 144
= 18.72mg

Total = 20.54 + 19.5 +19.5 +19.24 + 18.72
= **97.5mg**

For Diabetic + 200mg/kg B/w *Z. zanthoxyloides*

Head = 140g

Xmg - 140g

Xmg = 0.13 x 140
=18.2mg

Neck = 142g
Xmg - 142
Xmg = 0.13 x 142g
= 18.46mg

Body = 140.5g
Xmg - 140.5
Xmg = 0.13 x 140.5g
= 18.27mg

Tail = 140g
Xmg - 140g
Xmg = 0.13 x 140g
= 18.2mg

Plain = 140g
Xmg - 140g
Xmg = 0.13 x 144
= 18.2mg

Total = 18.2 + 18.46 + 18.27 + 18.2 + 18.2

= **91.32mg**

For Diabetic + 400mg/kg B/w *Z. zanthoxyloides*

Head = 165g

Xmg - 165g

Xmg = 0.13 x 165
= 21.45mg

Neck = 153g
Xmg - 153
Xmg = 0.13 x 153g
= 19.89mg

Body = 128g
Xmg - 128
Xmg = 0.13 x 128g
= 16.64mg

Tail = 160g
Xmg - 160g
Xmg = 0.13 x 160g
= 20.8mg

Plain = 158g
Xmg - 158g
Xmg = 0.13 x 158
= 20.54mg

Total = 21.45 + 16.64 + 20.8 +20.54
= **99.32mg**

For Diabetic + 5mg/kg B/w Standard Drug (Glibenclamide)

Head = 173g

Xmg - 173g

Xmg = 0.13 x 173
= 22.49mg

Neck = 155g
Xmg - 155g
Xmg = 0.13 x 155g
= 20.15mg

Body = 165g
Xmg - 165g
Xmg = 0.13 x 165g

= 21.45mg

Tail = 120g
Xmg - 120g
Xmg = 0.13 x 120g
= 15.6mg

Plain = 160g
Xmg - 160g
Xmg = 0.13 x 160
= 20.8mg

Total = 22.49 + 21.45 + 15.6 + 20.8
= **100.49mg**

Overall Total from Alloxan Concentration

Diabetic Control	=	114.73mg
Diabetic + 100mg/kg B/w Z.zanthoxyloides	=	97.5mg
Diabetic + 200mg/kg B/w Z.zanthoxyloides	=	91.32mg
Diabetic + 400mg/kg B/w Z.zanthoxyloides	=	99.32mg
Diabetic + 5mg/kg B/w Standard Drug	=	100.49mg

Total = 503.36mg

≈ 0.5g

Thus, dissolving alloxan in normal saline in the ratio 4:10 i.e. 4g of alloxan per 100ml of normal saline gives;

If 4g Alloxan - 100ml normal saline

Then 0.5g - Xml normal saline

Xml - $\frac{100}{4}$ x 0.5

= 25 x 0.5
= 12.5ml

Since 0.5g is small to be weighed and 12.5ml will not be enough for total administration,

then;

If 0.5g - 12.5ml of normal saline

Then 1.0g - Xml of normal soline

$$Xml = \frac{12.5}{0.5} \times 1$$

$$= 25 \times 1$$

$$= 25ml$$

;. 1g of alloxan will be dissolved in 25ml of normal saline.

If 4g of Alloxan = 100ml

Then Xg of Alloxan - 0.5ml

$$Xg - \frac{4}{100} \times 0.5$$

$$= 0.02g$$

Then if 0.02g – 150g rat

Then Xg – 1000g rat

$$Xg = \frac{0.02}{150} \times 1000$$

$$= 0.133g/1000g$$

$$\approx \mathbf{133mg/kg}$$

ADMINISTRATION OF TREATMENT ON ANIMAL SAMPLES

For 100mg/kg

If 1200mg - 80ml

Then Head = 15.8mg - Xml

$$\text{Xml} = \frac{80}{1200} \times \frac{15.8}{1}$$

$= 0.07 \times 15.8 = 1.05\text{ml}$

Neck = 15.0mg - Xml

$= 0.07 \times 15 = 1.05\text{ml}$

Body = 15.0mg - Xml

$= 0.07 \times 15 = 1.05\text{ml}$

Tail = 14.8mg - Xml

$= 0.07 \times 14.8 = 1.03\text{ml}$

Plain = 14.4mg - Xml

$= 0.07 \times 14.4 = 1.0\text{ml}$

For 200mg/kg

If 2248mg – 80ml

Then Head = 28mg - Xml

$$\text{Xml} = \frac{80}{2248} \times \frac{28}{1}$$

$= 0.036 \times 28$

$= 0.99\text{ml}$

Neck = 0.036×28.1

$= 1.01\text{ml}$

Body = 0.036×28.4

$= 1.00\text{ml}$

Tail = 0.036×28

= 0.99ml

Plain = 0.036 x 28

= 0.99ml

For 400mg/kg

If 4889.6mg - 80ml

Then Head = 66mg - Xml

$$Xml = \frac{80}{4889.6} \times \frac{66}{1}$$

Neck = 0.0163 x 61.2

= 1.0ml

Body = 0.0163 x 51.2

= 0.83ml

Tail = 0.0163 x 64

= 1.04ml

Plain = 0.0163 x 63.2

= 1.03ml

For Standard Drug (Glibendamibe)

If 5mg = 191.38ml

Then for Head which is 173g

= 0.02941mg (Conc. of treatment per day) – Xml

$$Xml = \frac{0.02941 \times 191.38}{5}$$

= 0.02941 x 38.28

= 1.13ml

For Neck which is 155g

= 0.026mg – Xml

Xml = 0.026 x 38.28

= 0.96ml

For Body which is 165g

= 0.028mg - Xml

Xml = 0.028 x 38.28

= 1.07ml

For Tail which is 120g

= 0.0204mg – Xml

Xml = 0.0204 x 38.28

= 0.78ml

For Plain which is 160g

= 0.0272mg - Xml

Xml = 0.0272 x 38.28

= 1.04ml

PREPARATION OF ALLOXAN FOR INDUCTION OF DIABETES ON ANIMAL SAMPLES

For Diabetic Control

If 1g - 25ml

Then 1000 mg – 25ml

For Head

23.79mg - Xml (23.79mg is the conc of alloxan in g/B/w)

$$Xml = \frac{25}{1000} \times 23.79$$

$= 0.025 \times 23.79$

$= 0.59ml$

Neck

22.95mg - Xml

$Xml = 0.025 \times 22.95$

$= 0.57ml$

Body

19.5mg - Xml

$Xml = 0.025 \times 19.5$

$= 0.439ml$

Tail

23.14mg - Xml

$Xml - 0.025 \times 23.14$

$= 0.58ml$

Plain

25. 35mg – Xml

$Xml – 0.025 \times 25 .35$

$= 0.63ml$

Total = 0.59 + 0.57 + 0.49 + 0.58 + 0.63 = **2.86ml**

Diabetic + 100mg/km B/w *Z. zanthoxyloides*

Head

20.54mg - Xml

Xml = 0.025 x 20.54

= 0.51ml

Neck

18.46mg - Xml

Xml = 0.025 x 18.46

= 0.46ml

Body

19.5mg - Xml

Xml = 0.025 x 19.5

= 0.49ml

Tail

19.24mg - Xml

Xml - 0.025 x 19.24

= 0.48ml

Plain

18.72mg - Xml

Xml – 0.025 x 18.72

= 0.47ml

Total = 0.51 + 0.46 + 0.9 + 0.48 + 0.47 = **2.41ml**

Diabetic + 200mg/km B/w *Z. zanthoxyloides*

Head

18.2mg - Xml

Xml = 0.025 x 18.2

= 0.46ml

Neck

18.46mg - Xml

Xml = 0.025 x 18.46

= 0.46ml

Body

18.27mg - Xml

Xml = 0.025 x 18.46

= 0.46ml

Tail

18.2mg - Xml

Xml - 0.025 x 18 .2

= 0.46ml

Plain

18. 2mg - Xml

Xml – 0.025 x 18 .2

= 0.46ml

Total = 0.46 + 0.46 + 0.46 + 0.46 + 0.46 = **2.3ml**

Diabetic + 400mg/kg B/w *Z. zanthoxyloides*

Head

21.45mg - Xml

Xml = 0.025 x 21.45

= 0.53ml

Neck

19.89mg - Xml

Xml = 0.025 X 19.89

= 0.49ml

Body

16.64mg - Xml

Xml = 0.025 x 16.64

= 0.4ml

Tail

20.8mg - Xml

Xml - 0.025 x 20.8

= 0.52ml

Plain

20. 54mg - Xml

Xml – 0.025 x 20.54

= 0.5135

Total = 0.53 + 0.49 + 0.4 + 0.52 + 0.51 = **2.45ml**

Diabetic + 5mg/kg B/w Standard Drug (Glibenclamide)

Head

22.49mg - Xml

Xml = 0.025 x 22.49

= 0.56ml

Neck

20.15mg - Xml

Xml = 0.025 x 20.15

= 0.50ml

Body

21.45mg - Xml

Xml = 0.025 x 21. 45

= 0.54ml

Tail

15.6mg - Xml

Xml - 0.025 x 15.6

= 0.4ml

Plain

20. 8mg - Xml

Xml - 0.025 x 20.8

= 0.52ml

Total = 0.56 + 0.50 + 0.54 + 0.04 + 0.52 = **2.52ml**

GRAND TOTAL = DC = 2.86ml

D + 100mg *Z. zanthoxyloides* = 2.41ml

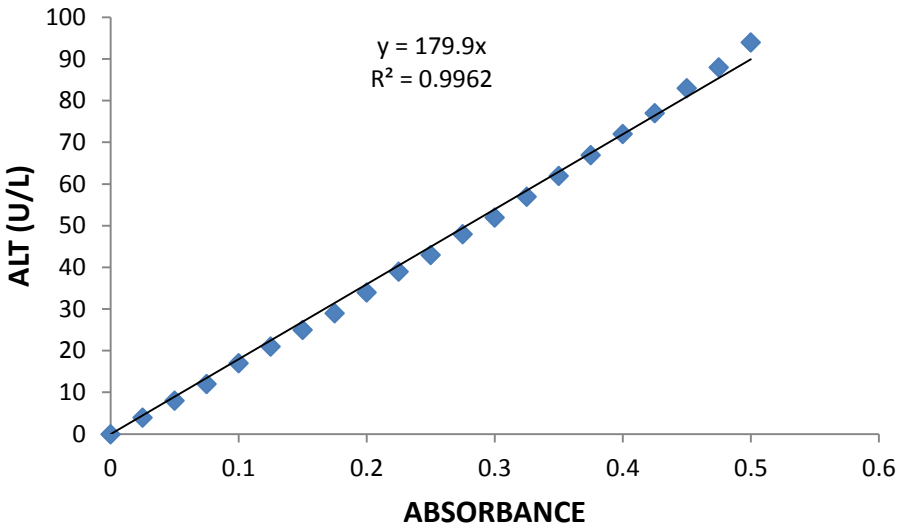
D + 200mg *Z. zanthoxyloides* = 2.3ml

D + 400mg *Z. zanthoxyloides* = 2.45ml

D + 5mg/kg S.D = 2.52ml

= **12.54ml**

CALIBRATION CURVE FOR ALANINE AMINOTRANSFERASE



CALIBRATION CURVE FOR ASPARTATE AMINOTRANSFERASE