EFFECT OF *Enantia chlorantha* STEM BARK EXTRACT ON THE HISTOPATHOLOGICAL LIVER OF STREPTOZOTOCIN-INDUCED DIABETIC IN RATS

BY

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DEPARTMENT OF BIOCHEMISTRY

FACULTY OF LIFE SCIENCE

UNIVERSITY OF BENIN

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A PROJECT SUMITTED TO THE DEPARTMENT OF BIOCHEMISTRY, FACULTY OF LIFE SCIENCE, IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCE, BSc. (HONS) BIOCHEMISTRY, OF THE UNIVERSITY OF BENIN, BENIN CITY.

JULY, 2021

CERTIFICATION

This is to certify that this project work was carried out by Omonkhegbele Precious Otibhor in the Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City under my supervision.

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DEDICATION

This work is dedicated to God almighty, my late grandmother Princess Grace Otiti Omonkhegbele- your baby is making you proud and bringing the dreams to reality and to my Parents Pastor Lawrence Akhere Omonkhegbele and Deaconess Adeola Betty Omonkhegbele

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I love you all

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ABSTRACT

Medicinal plants are plants that generally contain constituents that have been found useful for the treatment and management of both animal and human diseases. Diabetes mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both. This present work determined the ameliorative effect of the histopathological changes in Streptozotocin-Induced diabetic rats. Male wistar rats were purchased of age (16weeks), kept in clean and serene cages and left to acclimatize for two weeks and were fed with normal poultry feed before inducing them with Streptozotocin according to their weight and were divided into 7 groups and were administered treatment using Metformin, crude extract, ethyl acetate of 200mg and 400mg respectively. From the histopathological studies of the liver, it showed that the control group had hepatocytes and central vein organized, orderly and patterns are regular. The negative control group showed periportal hepatitis, inflammation of the hepatocytes and zonal necrosis while the group treated using Metformin showed a little improvement but there was still portal hepatitis and necrosis. When 200mg of the crude extract was administered, necrosis was reduced, inflammation persisted but mild. 400mg of the crude extract ameliorated the disease, no inflammation, no congestion, no necrosis, fine hepatocytes, Sinosoids were seen properly and Kupffer cells were activated. 200mg ethyl acetate tried in ameliorating the effects of the Streptozotocin damage. Small inflammation was observed, small congestion was observed , necrosis were really reduced and there was Kupffer cell activation too. 400mg ethyl acetate was administered and the inflammation though mild became more obvious compared to the 200mg of ethyl acetate which did a better job at ameliorating the effects of the Streptozotocin. The bile ducts were obvious and no congestion was observed.

CHAPTER ONE

1.0 INTRODUCTION

Medicinal plants are plants that generally contain constituents that have been found useful for the treatment and management of both animal and human diseases. Natural products from plants can be another potent source for the discovery of excellent activities such as blood booster, antioxidant, anti-ulcer, anti-cancer, antimicrobial etc (Omoregie *et al.* 2016).

Over 500 plants are known to be useful for medicinal purposes in Africa, but only a few have been described or studied (Taylor *et al.,* 2001). Natural products from plants can be another potent source for the discovery of excellent activities such as blood booster, antioxidant, anti-ulcer, anticancer, antimicrobial etc. The World Health Organization (WHO) emphasized the importance of scientific research into herbal medicine. Many developing countries of the world look upon native medicinal plants as possible addition to the WHO's list of "essential drugs" once their value have been clinically proven. Our survival and continued existence in turn depends on the efficiency with which man, with all the resources and technology available to him, harnesses, develops, utilizes plants and plant products (Ayoka *et al.,* 2008).

Diabetes Mellitus is an ancient medical term which early physicians used to designate a mysterious disease characterized by profuse, sweet-tasting urine. As medicine progressed the meaning of the term changed considerably. Unfortunately, the concept that diabetes mellitus is a disease-a distinct pathological entity has persisted and has caused much confusion both among the general public and within the health care professions. In fact, diabetes is a highly complex phenomenon which defies any simple explanation. It is clear, however, that diabetes is a major health problem which causes both premature death and major morbidity including blindness, kidney failure, premature cardiovascular disease, and gangrene of the lower extremities (Zimmet et al., 2001). Diabetes

mellitus is a metabolic disorder resulting from a defect in insulin secretion, insulin action or both. The condition itself introduces a need for patient's lifestyle adjustment to the disease and a number of everyday therapeutic and diagnostic restrictions. The main indication of diabetes mellitus is a hyperglycemia in blood which is due to inappropriate pancreatic insulin secretion or low insulin-directed fostering of glucose by target cells. Classification of diabetes mellitus is based on its etiology and clinical presentation. As such, there are four types or classes of diabetes mellitus viz; type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific types (Sicree *et al.*, 2006).

Streptozotocin is a permanent diabetes inducing drug. It is synthesized by a strain of the soil microbe *Streptomyces achromogenes* (gram positive bacterium) with broad spectrum of antibacterial properties (Dolan, 1997). Streptozotocin is an unusual aminoglycoside containing a nitrosoamino group discovered in 1959 as an antibiotic, now marketed as a generic drug. The nitrosoamino group enables the metabolite to act as a nitric oxide (NO) donor. NO is an important messenger molecule involved in many physiological and pathological processes in the body. Streptozotocin is widely used to induce diabetes in rodent models by inhibition of β-cell O-GlcNAcase (Vivek, 2010).

The African rainforest encompasses approximately 10 countries in west and central Africa, and is home to one of the greatest global diversity of fauna and flora in the world. This flora comprises many plants of medicinal value among them, there is Enantia chlorantha Oliver (or Annickia chlorantha). this plant is commonly known as African yellow wood and is also called Awopa, Osu pupa or Dokitaigbo (Yoruba), Osomolu (Ikale), Erumeru (Nigeria), Kakerim (Boki), Erenba-vbogo (Benin) , yellow moambi (English) and moambi jaune (French). It is an ornamental tree which may grow up to 30 m high with dense foliage and spreading crown . The outer bark which is thin and dark brown is fissured geometrically, while the inner bark is brown above and pale

cream beneath. The bark of Enantia chlorantha has several medicinal properties and has been used by traditional medical practitioners in Nigeria for the treatment of skin, gastric and duodenal ulcers, and as an antimalarial (Oliver *et al.*, 2016).

1.1 LITERATURE REVIEW

1.2.1 DIABETES

Diabetes, often referred to by doctors as diabetes mellitus, describes a group of metabolic diseases in which the person has high blood glucose (blood sugar), either because insulin production is inadequate, or because the body's cells do not respond properly to insulin, or both. Patients with high blood sugar will typically experience polyuria (frequent urination), they will become increasingly thirsty (polydipsia) and hungry (polyphagia). The meaning and origin of diabetes mellitus: Diabetes comes from Greek, and it means a "siphon". Aretus the Cappadocian, a Greek physician during the second century A.D., named the condition diabainein. He described patients who were passing too much water (polyuria) - like a siphon. The word became "diabetes" from the English adoption of the Medieval Latin diabetes. In 1675, Thomas Willis added mellitus to the term, although it is commonly referred to simply as diabetes. Mel in Latin means "honey"; the urine and blood of people with diabetes has excess glucose, and glucose is sweet like honey. Diabetes mellitus could literally mean "siphoning off sweet water".

It is characterized by the presence of hyperglycemia due to defective insulin secretion, defective insulin action or both. The chronic hyperglycemia of diabetes is associated with relatively specific long-term microvascular complications affecting the eyes, kidneys and nerves, as well as an increased risk for cardiovascular disease (CVD). The diagnostic criteria for diabetes are based on

thresholds of glycemia that are associated with microvascular disease, especially retinopathy (Roland and Zubin, 2013).

Diabetes may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss. The most severe clinical manifestations are ketoacidosis or a non-ketotic hyperosmolar state that may lead to dehydration, coma and, in the absence of effective treatment, death.

1.1.2 OVERVIEW OF DIABETES

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the pancreatic b-cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action. Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia.

Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of

atherosclerotic cardiovascular, peripheral arterial, and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes

1.1.3 TYPES OF DIABETES

The vast majority of cases of diabetes fall into two broad etiopathogenetic categories (discussed in greater detail below). In one category, type 1 diabetes, the cause is an absolute deficiency of insulin secretion. Individuals at increased risk of developing this type of diabetes can often be identified by serological evidence of an autoimmune pathologic process occurring in the pancreatic islets and by genetic markers (American Diabetes Association, 2014). In the other, much more prevalent category, type 2 diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response. In the latter category, a degree of hyperglycemia sufficient to cause pathologic and functional changes in various target tissues, but without clinical symptoms, may be present for a long period of time before diabetes is detected. During this asymptomatic period, it is possible to demonstrate an abnormality in carbohydrate metabolism by measurement of plasma glucose in the fasting state (American Diabetes Association, 2014).

1.1.3.1 TYPE 1 DIABETES (IMMUNE-MEDIATED DIABETES)

An autoimmune disease in which the immune system mistakenly destroys the insulin-making beta cells of the pancreas. It typically develops more quickly than other forms of diabetes. It is usually diagnosed in children and adolescents, and sometimes in young adults. To survive, patients must administer insulin medication regularly. Type 1 diabetes used to be called juvenile diabetes and insulin-dependent diabetes mellitus (IDDM). However, those terms are not accurate because children can develop other forms of diabetes, adults sometimes develop type 1, and other forms of diabetes can require insulin therapy. A variation of type 1 that develops later in life, usually after

age 30, is called latent autoimmune diabetes of adulthood (LADA). Sometimes patients with autoimmune diabetes develop insulin resistance because of weight gain or genetic factors. This condition is known as double diabetes (Samreen, 2009).

b-cell destruction is quite variable, being rapid in some individuals (mainly infants and children) and slow in others (mainly adults). Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others have modest fasting hyperglycemia that can rapidly change to severe hyperglycemia and/or ketoacidosis in the presence of infection or other stress. Still others, particularly adults, may retain residual b-cell function sufficient to prevent ketoacidosis for many years; such individuals eventually become dependent on insulin for survival and are at risk for ketoacidosis. At this latter stage of the disease, there is little or no insulin secretion, as manifested by low or undetectable levels of plasma C-peptide (Tobias et al., 2012).

Symptoms of diabetes type 1 includes;

- Weight loss-Insufficient insulin prevents the body from getting glucose from blood into cells to use as energy. The body starts burning fat and muscle for energy, causing reduction in weight
- Fatigue
- Frequent headaches due to high sugars
- Excessive hunger
- Chest burns & Indigestion-When stomach can't empty quickly enough (gastroparesis), unpleasant abdominal problems like nausea, vomiting, bloating, heartburn or feeling of fullness right after eating or long time afterwards experienced

- Excessive thirst & frequent urination-Blood sugar rises, the kidneys cannot retain extra sugar which is dumped into the urine, increasing urination & causes dehydration. Significant loss of potassium & other salts in excessive urination
- Breathlessness when exercising, including walking

1.1.3.2 TYPE 2 DIABETES (IMMUNE-MEDIATED DIABETES)

Type 2 diabetes mellitus (T2DM) is a metabolic disorder and typically results from excess of caloric intake over energy expenditure. It is characterized by a progressive insulin secretory defect due to insulin resistance, which increases the body's demand for insulin in order to retain glucose homeostasis. If pancreatic β-cells fail to secrete enough insulin to compensate for increasing insulin demand, the blood glucose level will be elevated gradually (Zhao et al., 2015). Chronic hyperglycemia is associated with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart, and blood vessels resulting in increasing levels of morbidity and mortality (Joy et al., 2013). T2DM associated with poor lifestyle is a primarily factor leading to the progressive reduction of physical activity and changes of dietary habits. As a consequence, a greater percentage of the population will become overweight and obese. T2DM is the one of the most prevalent chronic diseases worldwide and one of the major public health challenges of the 21st century. The vast majority of patients with diabetes suffer from T2DM (American Diabetes Association, 2010).

1.1.3.3 GESTATIONAL DIABETES

This type affects females during pregnancy. Some women have very high levels of glucose in their blood, and their bodies are unable to produce enough insulin to transport all of the glucose into their cells, resulting in progressively rising levels of glucose. Diagnosis of gestational diabetes is made during pregnancy. The majority of gestational diabetes patients can control their diabetes

with exercise and diet. Between 10 to 20 percent of them will need to take some kind of blood-glucose-controlling medications. Undiagnosed or uncontrolled gestational diabetes can raise the risk of complications during childbirth (Piero *et al.,* 2014).

1.1.3.4 SECONDARY DIABETES

Diabetes caused by another condition. The many potential sources of secondary diabetes range from diseases such as pancreatitis, cystic fibrosis, Down syndrome and hemochromatosis to medical treatments including corticosteroids, other immunosuppressives, diuretics and pancreatectomy (Samreen, 2009).

1.1.4 PREVALENCE OF DIABETES

The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. Quantifying the prevalence of diabetes and the number of people affected by diabetes, now and in the future, is important to allow rational planning and allocation of resources. Globally, diabetes prevalence is similar in men and women but it is slightly higher in men <60 years of age and in women at older ages. Overall, diabetes prevalence is higher in men, but there are more women with diabetes than men (data available from the authors). The combined effect of a greater number of elderly women than men in most populations and the increasing prevalence of diabetes with age is the most likely explanation for this observation (Amos, 1997).

In developing countries, the majority of people with diabetes are in the 45- to 64-year age range, similar to the finding reported previously (2). In contrast, the majority of people with diabetes in developed countries are >64 years of age. By 2030, it is estimated that the number of people with diabetes >64 years of age will be >82 million in developing countries and >48 million in developed countries (Sarah et al., 2004).

1.1.5 EPIDEMIOLOGY OF DIABETES

The disease burden related to diabetes is high and rising in every country, fueled by the global rise in the prevalence of obesity and unhealthy lifestyles. The latest estimates show a global prevalence of 382 million people with diabetes in 2013, expected to rise to 592 million by 2035. The aetiological classification of diabetes has now been widely accepted. Type 1 and type 2 diabetes are the two main types, with type 2 diabetes accounting for the majority (>85%) of total diabetes prevalence. Both forms of diabetes can lead to multisystem complications of microvascular endpoints, including retinopathy, nephropathy and neuropathy, and macrovascular endpoints including ischaemic heart disease, stroke and peripheral vascular disease (Hypponen et al., 2001).

The incidence of type 1 diabetes in children varies nearly 400- fold between countries with age-adjusted incidence rates ranging from 0.1 per 100,000 per year in parts of Venezuela and China to 37.8 in Sardinia and 40.9 per 100,000/year in Finland (Diamond Project Group, 2006). The high rate observed in Sardinia is notably discordant with the incidence in Italy as a whole. Incidence also varies within several other countries including China, where there is a 12-fold variation by region. In general, countries in Europe and North America have either high or intermediate incidences. The incidence in Africa is generally intermediate and that in Asia is low, with the notable exception of Kuwait (Gale and Gillespie, 2001).

Type 1 diabetes can occur at any age, but in most populations the incidence is highest between birth and 14 years old. Generally, all populations display a steady increase in incidence rate with age up to around 10e15 years. There are no population-based incidence data for ages above 35 years. Overall, there is a slight male excess among children in high-incidence countries, while the opposite is seen in low-incidence countries, but these differences are small. However, there is

generally a male excess among young adults, and peak incidence is around puberty in most populations (Gale and Gillespie, 2001).

Genetic susceptibility is important but not sufficient in causation of type 1 diabetes. Environmental factors have a more important role in progression from islet autoimmunity to overt disease, possibly because improved living standards have reduced microorganism exposure, leading to increased autoimmunity. Case-control studies have shown associations with early social mixing, viral infections, toxins and dietary factors such as exclusive breast-feeding and delayed introduction of cows' milk. Hypothesized aetiological factors include a vitamin D deficiency (Hypponen et al., 2001).

The slow onset of type 2 diabetes, and its usual presentation without the acute metabolic disturbance seen in type 1 diabetes, means that the true time of onset is difficult to determine. There is also a long pre-detection period, and up to one-half of cases in the population may be undiagnosed.

The main pathophysiological defects leading to type 2 diabetes are insulin resistance and a relative insulin secretory defect. The main aetiological risk factors are age, obesity, family history, and physical inactivity. Dietary risk factors have recently emerged: risk is increased by high consumption of red and processed meat (Bendinelli, 2012) and sugar-sweetened beverages, and reduced by intake of fruit and vegetables, some types of dairy products, and some overall dietary patterns. It is likely that interactions between the environment/lifestyle and genetic factors provide the explanation for the risk of type 2 diabetes, but demonstrating such interaction is challenging. Encouraging research findings have recently shown higher absolute risk of diabetes associated with obesity at any level of genetic risk (Langenberg et al., 2014).

1.1.6 PATHOPHYSIOLOGY OF DIABETES

The autoimmune destruction of pancreatic β-cells, leads to a deficiency of insulin secretion which results in the metabolic derangements associated with IDDM. In addition to the loss of insulin secretion, the function of pancreatic α-cells is also abnormal and there is excessive secretion of glucagons in IDDM patients. Normally, hyperglycemia leads to reduced glucagons secretion, however, in patients with IDDM, glucagons secretion is not suppressed by hyperglycemia (Raju and Raju, 2010). The resultant inappropriately elevated glucagons levels exacerbate the metabolic defects due to insulin deficiency. The most pronounced example of this metabolic disruption is that patients with IDDM rapidly develop diabetic ketoacidosis in the absence of insulin administration. Although insulin deficiency is the primary defect in IDDM, there is also a defect in the administration of insulin. There are multiple biochemical mechanisms that account for impairment of tissue's response to insulin. Deficiency in insulin leads to uncontrolled lipolysis and elevated levels of free fatty acids in the plasma, which suppresses glucose metabolism in peripheral tissues such as skeletal muscle (Raju and Raju, 2010). This impairs glucose utilization and insulin deficiency also decreases the expression of a number of genes necessary for target tissues to respond normally to insulin such as glucokinase in liver and the GLUT 4 class of glucose transporters in adipose tissue. The major metabolic derangements, which result from insulin deficiency in IDDM are impaired glucose, lipid and protein metabolism (Raju and Raju, 2010).

Individuals with NIDDM have detectable levels of circulating insulin, unlike patients with IDDM and the pathophysiology of type 2 diabetes is described in Figure 3. On the basis of oral glucose tolerance testing the essential elements of NIDDM can be divided into four distinct groups:

i) Those with normal glucose tolerance.

ii) Chemical diabetes (called impaired glucose tolerance).

iii) Diabetes with minimal fasting hyperglycemia (fasting plasma glucose less than 140 mg/dl).

iv) Diabetes mellitus in association with overt fasting hyperglycemia (fasting plasma glucose greater than 140 mg/dl).

The individuals with impaired glucose tolerance have hyperglycemia inspite of having highest levels of plasma insulin, indicating that they are resistant to the action of insulin. In the progression from impaired glucose tolerance to diabetes mellitus, the level of insulin declines indicating that patients with NIDDM have decreased insulin secretion. Insulin resistance and insulin deficiency are common in the average NIDDM patients (Holt, 2004). Insulin resistance is the primary cause of NIDDM, however some researcher contend that insulin deficiency is the primary cause because a moderate degree of insulin resistance is not sufficient to cause NIDDM (Raju and Raju, 2010). Most patients with the common form of NIDDM have both defects. Recent evidence has demonstrated a role for a member of the nuclear hormone receptor super family of proteins in the etiology of type 2 diabetes (Raju and Raju, 2010). Relatively new classes of drugs used to increase the sensitivity of the body to insulin are the thiazolidinedione drugs. These compounds bind to and alter the function of the peroxisome proliferators-activated receptor g (PPARg). PPARg is also a transcription factor and when activated, binds to another transcription factor known as the retinoid x receptor (RXR). When these two proteins are complexed a specific set of genes becomes activated. PPARg is a key regulator of adipocyte differentiation; it can induce the differentiation of fibroblasts or other undifferentiated cells into mature fat cells. PPARg is also involved in the synthesis of biologically active compounds from vascular endothelial cells and immune cells (Raju and Raju, 2010).

1.1.7 CAUSES OF DIABETES

Type 1 diabetes is an auto-immune disorder in which the immune system destroys tiny portion of the pancreatic tissue (The organ which produces the insulin hormone). It is diagnosed when 90% of beta-cells that produce insulin are destroyed. It is a genetic disease, but not hereditary. Those likely to develop type Diabetes have a gene mutation that causes the antibodies. Pancreas need enzyme Glutamic Acid Decarboxylase (GAD) to function normally. Antibodies that target this enzyme are called GAD antibodies. Other antibodies associated with type 1 diabetes include: i. Islet cell cytoplasmic autoantibodies (ICAs) ii. Insulinoma-associated-2 autoantibodies (IA-2As) iii. Insulin autoantibodies (IAAs) (more common in children than adults) The more autoantibodies, more aggressive are the autoimmune attack (Will, 2009). The genetic code for diabetes is present on one of the genes (chromosome 6). It was reported that the Human Leukocyte Antigen (HLA-DR) genes are responsible for causing Type 1 diabetes. But genes are like the loaded gun, it is the environmental factors which act as the trigger. For those who have the gene (HLA-DR), a trauma or viral infection (flu) triggers the onset. Rotavirus infects the pancreas by hijacking a receptor on pancreas cells, causing cell death. This infection is thought to trigger the autoimmune destruction of beta cells. Some other triggers include bacterial infection, unidentified components that cause autoimmune reaction, chemical toxins in food, underlying genetic disposition (Will, 2009).

In type 2 diabetes, the body does not produce enough insulin for proper function, or the cells in the body do not react to insulin (insulin resistance). Approximately 90% of all cases of diabetes worldwide are type 2. Some people may be able to control their type 2 diabetes symptoms by losing weight, following a healthy diet, doing plenty of exercise, and monitoring their blood glucose levels. However, type 2 diabetes is typically a progressive disease - it gradually gets worse - and the patient will probably end up have to take insulin, usually in tablet form. Overweight and

obese people have a much higher risk of developing type 2 diabetes compared to those with a healthy body weight. People with a lot of visceral fat, also known as central obesity, belly fat, or abdominal obesity, are especially at risk. Being overweight/obese causes the body to release chemicals that can destabilize the body's cardiovascular and metabolic systems. Being overweight, physically inactive and eating the wrong foods all contribute to our risk of developing type 2 diabetes. Those with a close relative who had/ had type 2 diabetes, people of Middle Eastern, African, or South Asian descent also have a higher risk of developing the disease. Men whose testosterone levels are low have been found to have a higher risk of developing type 2 diabetes (Piero *et al.*, 2014).

1.2 **STREPTOZOTOCIN (STZ)**

Streptozotocin is a permanent diabetes inducing drug. It is synthesized by a strain of the soil microbe *Streptomyces achromogenes* (gram positive bacterium) with broad spectrum of antibacterial properties (Dolan, 1997). Streptozotocin is an unusual aminoglycoside containing a nitrosoamino group discovered in 1959 as an antibiotic, now marketed as a generic drug. The nitrosoamino group enables the metabolite to act as a nitric oxide (NO) donor. NO is an important messenger molecule involved in many physiological and pathological processes in the body. Streptozotocin is widely used to induce diabetes in rodent models by inhibition of β-cell O-GlcNAcase (Vivek, 2010).

Streptozotocin features four important biological properties as evidenced by its antibiotic, β-cell (beta)-cytotoxic, oncolytic, as well as oncogenic effects. This product is an antineoplastic antibiotic and is used mainly in the treatment of pancreatic (islet cell) tumors. It is used for the treatment of malignant insulinoma. Current use of STZ is mostly as an investigational drug for

diabetes research due to its specific toxicity associated with pancreatic β-cells. Low affinity glucose transporter- GLUT2 of βcells transports STZ into the cell and causes alkylation of DNA and irreversible necrosis of β cells. DNA synthesis in mammalian and bacterial cells is inhibited by action of STZ . STZ is widely used to induce both insulin-dependent (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM). STZ is an antibiotic and antitumor agent, induces diabetes mellitus via reduction of nicotinamide adenine dinucleotide in pancreatic β-cells (Bolzan and Bianchi, 2002).

1.2.1 STRUCTURAL FEATURES OF STZ

Streptozotocin (2-deoxy-2-[3-methyl-3-nitrosourea] 1-D-glucopyranose) occurs in two anomeric forms, α and β (Figure1a), which can be separated by Chromatographic technique (HPLC). It appears as pale yellow or off-white crystalline powder. Streptozotocin has a molecular weight of 265 g/mol, with molecular formula $C_8H_{15}N_3O_7$. STZ molecular structure is similar to that of 2-deoxy-D-glucose with a replacement at C2 with an N-methyl-N-nitrosourea group, which is the cytotoxic moiety of STZ in damaging beta cells. Streptozotocin is a glucosamine nitrosourea compound with a methyl group attached at one end and a glucose molecule at the other end (Busineni *et al.*, 2015).

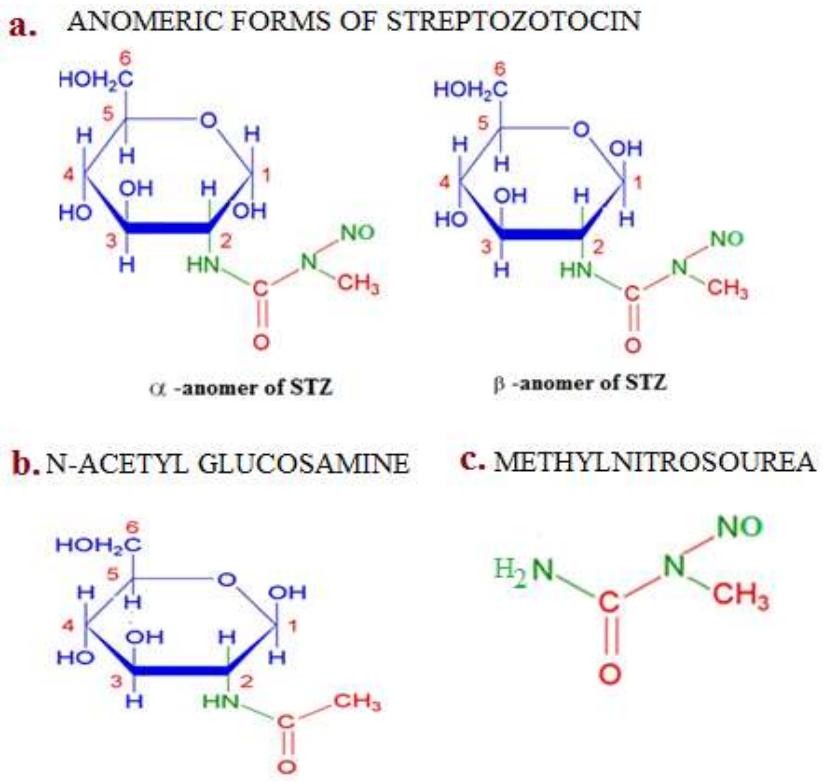

Figure 1.1: A schematic diagram representing - a) α and β Anomeric forms of STZb) structural analog

of STZ - N-acetyl glucosamine c) cytotoxic moiety of STZ- N-methyl-Nnitrosourea

1.2.2 MECHANISM OF ACTION OF STREPTOZOTOCIN

Despite its use to create animal models of diabetes for over several decades, the mode of action of STZ is not fully understood. Mode of action of STZ cellular toxicity includes its selective uptake by pancreatic β cells and its toxic effector mechanism in the animal body to create a diabetic model.

1.2.2.1 SELECTIVE TRANSPORTATION OF STREPTOZOTOCIN ACROSS B CELL MEMBRANES:

STZ toxic action involves its selective uptake into β cells via its low affinity glucose transporter GLUT2 present in the plasma membrane. Unlike the general lipophilic nature of its group compounds- nitrosoureas, STZ is hydrophilic in nature due to glucose moiety substitution. Hydrophilic nature of STZ limits its free radical diffusion across phospholipid bilayered plasma

membrane of cells because of their hydrophobicity. The 2deoxy glucose moiety of STZ allows its selective uptake into β cells through glucose transporter (GLUT)-2 due to its structural analogy with glucose. Hepatocytes and the renal tubular cells also express GLUT 2 transporter and are susceptible to STZ (Lenzen, 2008).

Non-β endocrine cells in pancreatic islets such as α- and δ-cell as well as the extra-pancreatic parenchyma remain intact after STZ challenge, indicating the beta cell selective properties of STZ. STZ also causes cardiac and adipose tissue damage and increases oxidative stress, inflammation, endothelial dysfunction with the concentrations of the drug or its metabolites in the liver, kidney, intestine and pancreas being consistently higher than those in the plasma. Once STZ enters into the cell, it inhibits the glucose metabolism and insulin secretion of beta cells and impairs the pancreas (Valentovic *et al.*, 2006).

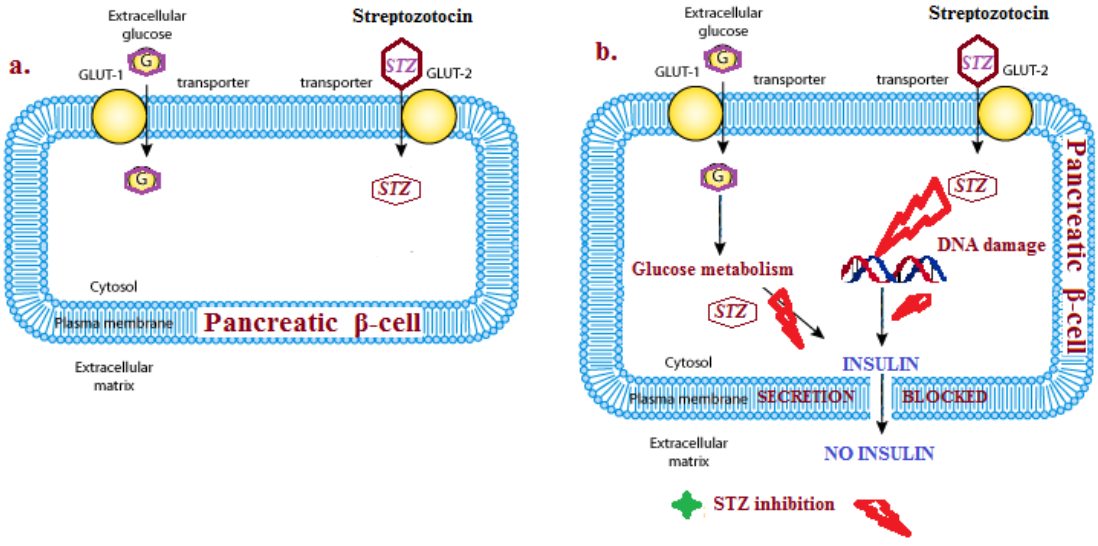

Figure 1.2: A schematic diagram representing – a)Model of STZ and glucose uptake by pancreatic beta cellsthrough selective transporters. b) Inhibition of glucose metabolism and inhibition of insulin secretion by STZ in pancreatic beta cells. Red marks represent the inactivation of the metabolic steps of insulin production by STZ.

1.2.2.2 EFFECTOR MECHANISM OF STZ TOXICITY

Proposed mechanism of β cellular toxicity of STZ includes –

1. Carbamoylation and alkylation of cellular components.
2. Release of nitric oxide (NO).
3. *Free Radicals*ee radical generation and Oxidative stress.
4. Inhibition of O-GlcNAcase.

1. Carbamoylation and alkylation of cellular components

Antibiotic STZ is a highly genotoxic alkylating agent, which can cause cellular damage including DNA strand breaks and will eventually lead to cell death. Once STZ enters inside the cell, it is able to decompose spontaneously to form an isocyanate molecule and a methyldiazohydroxide molecule (62, 63) (Figure3). The isocyanate can undergo intramolecular carbamolyation or can carbamoylate other cellular components like proteins. The methyldiazohydroxide can decompose to form a highly reactive carbonium ion (CH3+) which is believed to be a key player in STZ-induced DNA alkylation causing interstrand DNA cross-links (Busineni *et al.*, 2015).

2. Release of nitric oxide (NO)

Like other nitrosoureas STZ is a potential NO radical donor in in vivo conditions, that mediates the destruction of pancreatic β islet cells through DNA damage. After two hours of STZ injection NO release is observed in pancreatic β cells. In order to confirm that the NO formation was not attributed to nitric oxide synthtase (NOS), inhibitors of NOS used in the experiment did not block the NO formation generated in the presence of STZ (Wada and Yagihashi, 2004). NO release after STZ injection is evidenced by increased activity of guanyl cyclase and the formation of cGMP,

which are the characteristic features of NO in execution of its biological function. (Busineni *et al.*, 2015).

Variable low levels of free radical scavenging enzymes in pancreatic β-cells makes them more sensitive to cytotoxic action of NO radical. It still remains to be determined which are the intracellular targets for NO-induced damage. But available data supports that NO radical produced during STZ metabolism have the following possible intracellular targets, which makes the β-cell dysfunction and death. NO radical inactivates the mitochondrial enzyme aconitase, impairing substrate oxidation and ATP production. Ionic channels and complexes I and II of the mitochondrial electron transport chain are two other possible targets for NO effects which may impair insulin secretion. NO also leads to nuclear DNA damage in both rat and human pancreatic beta-cells, as evaluated by the 'comet assay' (Busineni *et al.*, 2015).

The effects of NO at the DNA level are complex, and involve formation of N-nitrosoamines. NO damages DNA in two ways that is by nitration of nucleic acids and deamination of purines and pyrimidines, or damage induced by peroxynitrite formation by reacting with super oxide free radical. Besides inducing over DNA damage, NO may also inactivate DNA repair/replication enzymes. The outcome of NO-induced beta-cell DNA damage can be cell death by apoptosis or, in some cases, necrosis (DorotaŠoltésová, and IvetaHerichová, 2011).

3. *Free Radicals*ee radical generation and oxidative stress

Reports say that there is a tissue-specific increase in oxidative stress with persistent increase in the generation of free radicals. Free radical production in the early stages of streptozotocin induced diabetic rats includes reactive oxygen and nitrogen species (ROS and RNS) like superoxide radical ($O2^{\circ-}$), hydroxyle radical ($OH^{\circ-}$), hydrogen peroxide ($H2O2$) and Peroxynitrite ($ONOO^-$), that

cause oxidative stress . Several studies have demonstrated that hyperglycemia in STZ induced diabetes has been associated with increased formation of reactive oxygen species (ROS) and oxidative damage to tissue compounds. Oxidative stress in STZ induced diabetic animals is due to glucose auto-oxidation, protein glycation, formation of advanced glycation products and the polyol pathway that generates free radicals (Busineni *et al.*, 2015).

4. Inhibition of O-GlcNAcase

STZ specifically kills islet cells by inhibiting O-GlcNAcase (OGA). O-GlcNAcase is a glycoside hydrolase that cleave the beta-O-linked GlcNAc (N-acetyl glucosamine (O-GlcNAc)) *Free Radicals*ommodified proteins in the cytosol of β-cell during protein posttranslational modification for the generation of good and safer functional proteins (Inhibition of OGA (OGlcNAcase) by STZ results in the hyper-O-GlcNAcylation (due to irreversible O- glycosylation), which cause the accumulation of harmful proteins and activation of stress pathways leading to apoptosis. The mode of inhibition involves covalent modification of the enzyme or the enzyme-catalyzed formation of a tight binding inhibitor (Liu *et al.*, 2000).

1.3 MEDICINAL PLANTS

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants consider as important source of nutrition and as a result of that these plants recommended for their therapeutic values. These plants include ginger, green tea, walnuts and some others plants. Other plants their derivatives consider as important source for active ingredients which are used in aspirin and toothpastes (Bassam, 2012). It has been estimated that about 13,000 species of plants have

been employed for at least a century as traditional medicines by various cultures around the world. A list of over 20,000 medicinal plants has been published, and very likely a much larger number of plants.

These days the term Alternative Medicine became very common in western culture, it focus on the idea of using the plants for medicinal purpose. But the current belief that medicines which come in capsules or pills are the only medicines that we can trust and use. Even so most of these pills and capsules we take and use during our daily life came from plants. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics and antimalaria medications, contain ingredients from plants (Yudharaj, 2016).

1.4.0 *Enantia chlorantha*

Fig: 1.3: E*nantia chlorantha*

The African rainforest encompasses approximately 10 countries in west and central Africa, and is home to one of the greatest global diversity of fauna and flora in the world. This flora comprises many plants of medicinal value among them, there is Enantia chlorantha Oliver (or Annickia chlorantha). Belonging to annonaceae family, this plant is commonly known as African yellow wood and is also called Awopa, Osu pupa or Dokitaigbo (Yoruba), Osomolu (Ikale), Erumeru

(Nigeria), Kakerim (Boki), Erenba-vbogo (Benin) , yellow moambi (English) and moambi jaune (French). It is an ornamental tree which may grow up to 30 m high with dense foliage and spreading crown . The outer bark which is thin and dark brown is fissured geometrically, while the inner bark is brown above and pale cream beneath (Olivier et al., 2016). The stem is fluted and aromatic while the elliptic leaves are about 0.14–0.15 m long and 0.05–0.14 m broad. The leaves display up to 20 pairs of prominent lateral vein and parallel secondary nerves. It is a dense forest tree found in the east and south forest of Cameroon, south part of Nigeria, Gabon, Angola and DRC. Enantia chlorantha is common in Cameroon and is a medicinal tree mostly used for the treatment of malaria and typhoid fever, but also used against other ailments of the human body. The bark, and in rare cases the leaves or the roots, are the main parts used for medicinal purposes (Iwu,1993).

1.4.1 MEDICINAL BENEFITS OF ENANTIA CHLORANTHA

In the traditional medicine, this plant has been used for a long time in many parts of the African continent to treat various ailments of the human body. A concoction Enantia Chlorantha has been used to treat malaria symptoms, aches, wounds, boils, vomiting, yellow bitter, fever, chills, sore, spleen in children (Tsabang et al., 2012). The stem bark decoction, taken orally, has also been reported to treat intestinal worms, intestinal spasms, malaria and sexual asthenia . The same decoction is effective against hepatitis, jaundice, urinary tract infections and typhoid fever (Jiofack et al., 2009). Root decoction is used for malaria, jaundice and as antipyretic. Dried stem bark is used to treat malaria, hepatic disorders, tuberculosis and ulcers. Gill and Akinwunmi noted the use of infusion of bark for the treatment of cough and wounds in Nigeria. In the southern forest zone of Cameroon, Enantia chlorantha is also used for the management of stomach problems, as well as for the treatment of jaundice, tuberculosis, urinary tract infections, malaria, hepatitis and some

forms of ulcer. In the western Cameroon, a mixture of Enantia chlorantha bark, cut into small pieces, Citrus limonum fruit also cut into small pieces with its peels and Allium sativum bulb crushed is macerated in water for two days and the resulting liquid taken twice daily to cure malaria (Olivier et al., 2016).

1.5 PHYTOCHEMISTRY

Phytochemicals are biologically active, naturally occurring chemical compounds found n plants, which provide help benefits for humans. They help plants thrive or thwart competitors, predators or pathogens. It has been shown that phytochemicals have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals are known and documented, and are classified by protective function, physical characteristics and chemical characteristics. Phytochemicals are found in fruits, vegetables, legumes, wholegrains, nuts, seeds, fungi, herbs and spices (Mathai, 2000).

Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds (Costa *et al.*, 1999). Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Phytochemicals are not essential nutrients and are not required by the human body for sustaining life, but have important properties to prevent or to fight some common diseases. Many of these benefits suggest a possible role for phytochemicals n the prevention and treatment of diseases.

There are different ways in which a phytochemical work;

- It can act as an antioxidant and protect cells against free radical damage e.g polyphenols, carotenoids, etc.

- It can stimulate certain enzymes, thereby reduce risk for breast cancer, e.g terpenes. It may act as an anti-bacterial and hormonal stimulant component.
- It also acts as binders which may prevent the adhesion of pathogens to the human cell walls.

Some very important phytochemicals include:

1.5.1 FLAVONOIDS:

Flavonoids are a class of plant and fungus secondary metabolites. They are polyphenolic compounds that are ubiquitous in nature. They occur in vegetables, fruits and beverages like tea, coffee, and fruit drinks. Chemically, flavonoids have the general structure of a 15- carbon skeleton, which consists of two phenyl rings (A and B) and a heterocyclic ring (C). This carbon structure has been abbreviated C6-C3-C6.

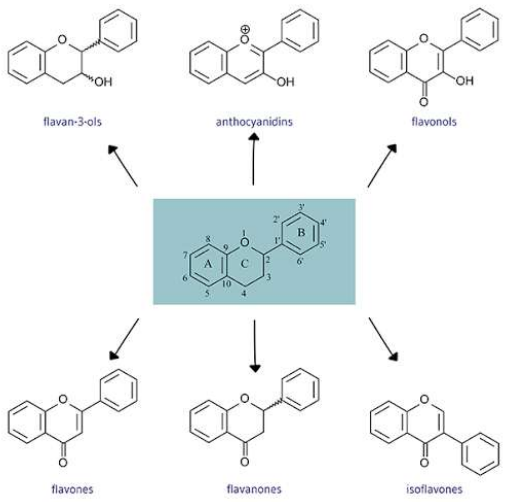

Fig.1.4: Structure of flavonoid subclasses.

Flavonoids are classified into:

Flavonoids or bioflavonoids

Isoflavonoids, derived from 3-phenyl chromen-4-one structure

Neoflavonoids, derived from 4-phenylcoumarine structure

Flavonoids have been reported to exert multiple biological property including anti-microbial, cytotoxicity, anti-inflammatory as well as anti-tumour activities. They have the capacity to act as powerful antioxidants which can protect the human body from free radicals and reactive oxygen species. Flavonoids possess many useful properties, enzyme inhibition, oestrogenic activity, anti-allergic activity, vascular activity and cytotoxic anti-tumour activity (Tapas *et al.*, 2008). Flavonoids play important roles in protecting biological systems against the harmful effects of oxidative processes on macromolecules, such as carbohydrates, proteins, lipids and DNA (Atmani *et al*., 2009).

1.5.2.2 TANNINS

Tannins are a heterogeneous group of high molecular weight polyphenolic compounds with the capacity to form reversible and irreversible complexes with proteins, polysaccharides (cellulose, hemicellulose, pectin etc), alkaloids, nucleic acids and minerals etc (Schofield *et al.*, 2001). There are four major classes of tannins

- Gallotannins
- Ellagitannins
- Complex tannins
- Condensed tannins.

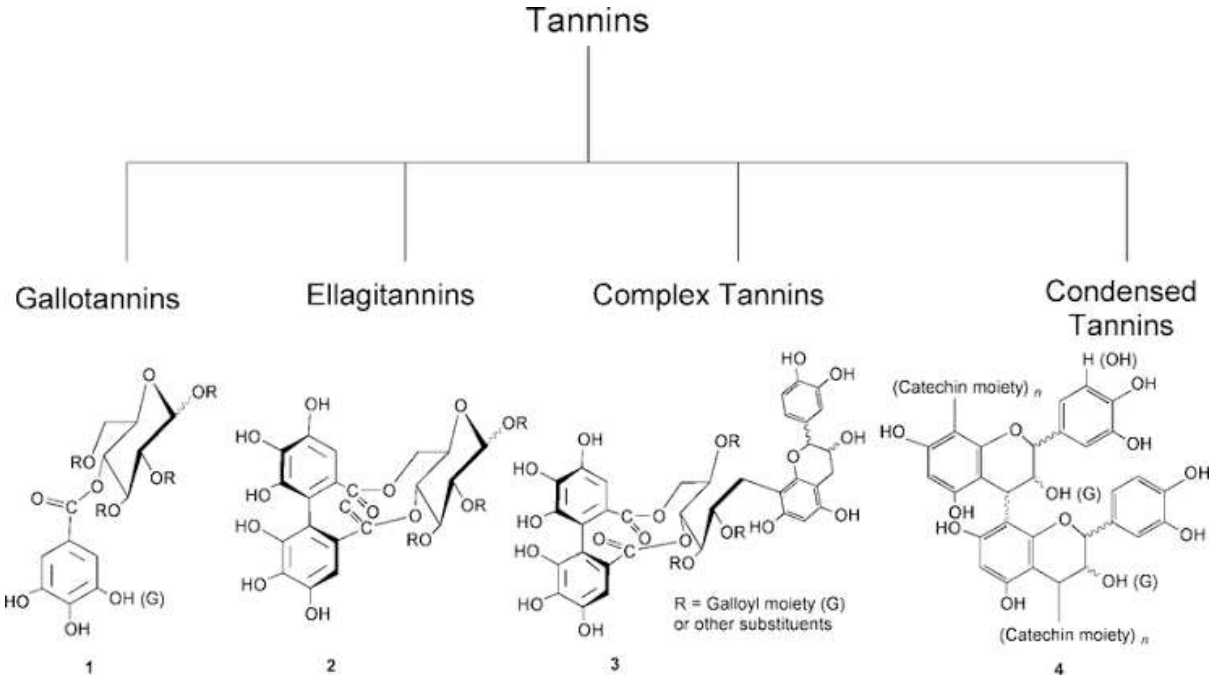

Fig.1.5: Structure of tannins.

Tannins are commonly found in fruits such as grapes, persimmon, blueberry, tea, chocolate, legume forages etc.

Tannin containing plant extracts are used as astringents, against diarrhoea, as diuretics against stomach and duodenal tumours and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals (Dolara *et al.*, 2005). Tannins are used in the dyestuff industry as caustics for cationic dyes and also in the production of inks. In the food industry, tannins are used to clarify wine, beer and fruit juices. Recently, tannins have attracted scientific interests, especially due to the increased incidence of deadly illnesses such as AIDS and various cancers.

1.5.2 ALKALOIDS:

Alkaloids are a class of naturally occurring organic compounds that mostly contain basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic

properties. In addition to carbon, nitrogen and hydrogen, alkaloids may also contain oxygen, sulphur and more rarely other elements such as chlorine, bromine and phosphorus.

Alkaloids are naturally synthesized by a large number of organisms, including animals, plants, bacteria and fungi. Alkaloids have a wide range of pharmacological activities including antimalarial (e.g quinine), antiasthma (e.g ephedrine), anticancer (e.g homoharringtonine), cholinomimetric (e.g galantamine), vasodilatory (e.g vincamine), antiarrhythmic (e.g quinidine), analgestic (e.g morphine), antibacterial (e.g chelerythrine), and antihyperglycemic activities (e.g piperidine).

Alkaloids are also used as local anaesthetic and stimulant as cocaine. Almost all alkaloids have a bitter taste.

The various classes of alkaloids according to the heterocyclic ring system they contain are :

- Pyrrolidine alkaloids- they contain pyrollidine ring system e.g hygrine
- Pyridine alkaloids- they have piperidine ring system e.g coniine, piperine
- Pyrollidine-pyridine alkaloids- they contain pyrollidine-pyridine rind system e.g myosmine, nicotine.
- Pyridine-piperidine alkaloids- they contain pyridine ring system joined to a piperidine ring system e.g anabasine
- Quinolone alkaloids- they have heterocyclic ring system quinolone e.g quinine
- Isoquinoline alkaloids- they contain the heterocyclic ring system isoquinoline e.g opium alkaloids like nicotine, morphine, codeine.

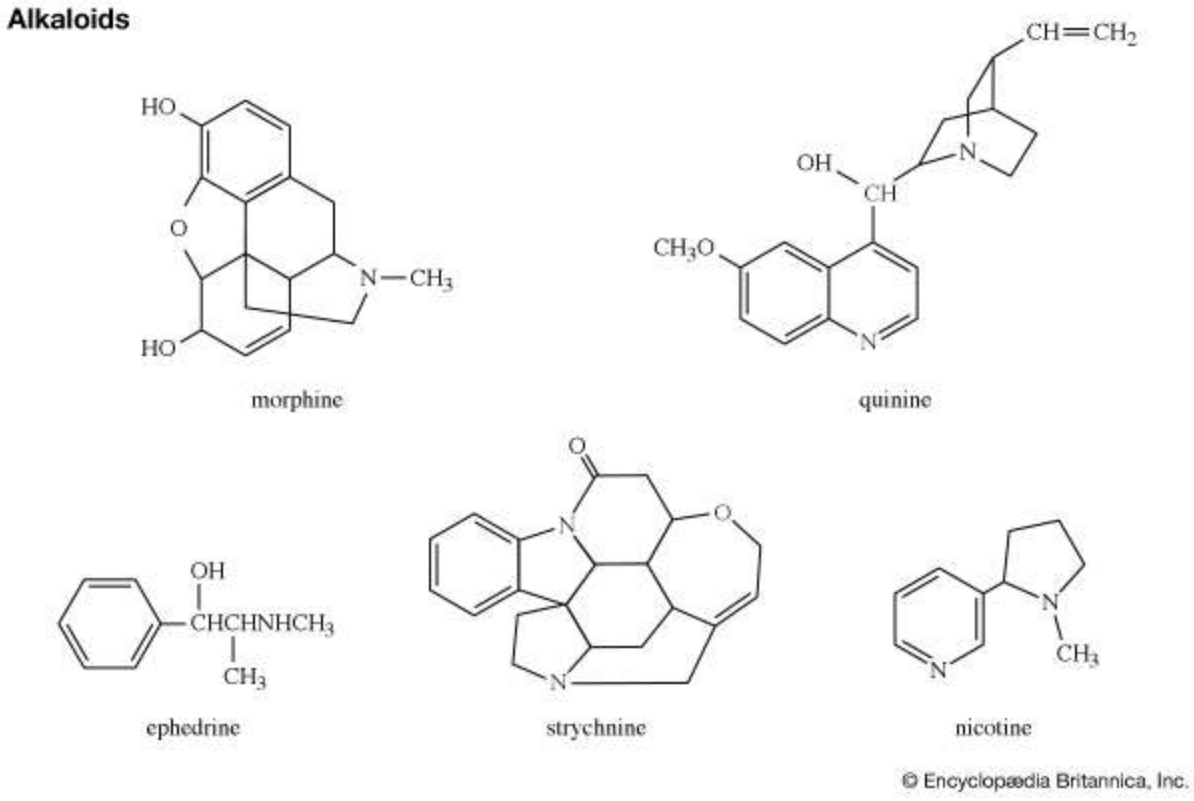

Fig.1.6: structure of Alkaloids.

Alkaloids are significant or the protecting and survival of plant as they ensure their survival against microorganisms, insects and herbivores and also against other plants by means of allelopathically active chemicals (Molyneux *et al.,* 1996). Some alkaloids have stimulant properties such as caffeine and nicotine.

1.5.3 TERPENOIDS:

Terpenoids are large and diverse class of naturally occuring organic chemicals derived from terpenes. They are derived from five carbon isoprene units. Terpenoids are used as flavours and fragrances n food and cosmetics e.g menthol and sclareol. They are also important for the quality of agricultural products, such as flavour or fruits and the fragrance of flowers like linalool. The building block of terpenoids is the hydrocarbon isoprene, CH2=C(CH3)-CH=CH2. They therefore

have the molecular formula (C5H8)n and are classified according to the isoprene units (Langenheim, 1994) as

- Hemiterpenoids- consist of a single isoprene unit
- Monoterpenoids- consists of two isoprene unit's e.g limonene, camphor
- Sesquiterpenes- have three isoprene units e.g artemisinin
- Diterpenes- consist of four isoprene units
- Triterpenes- consists of six isoprene units e.g lanosterol, squalene
- Tetraterpenoids- consists of eight isoprene units.

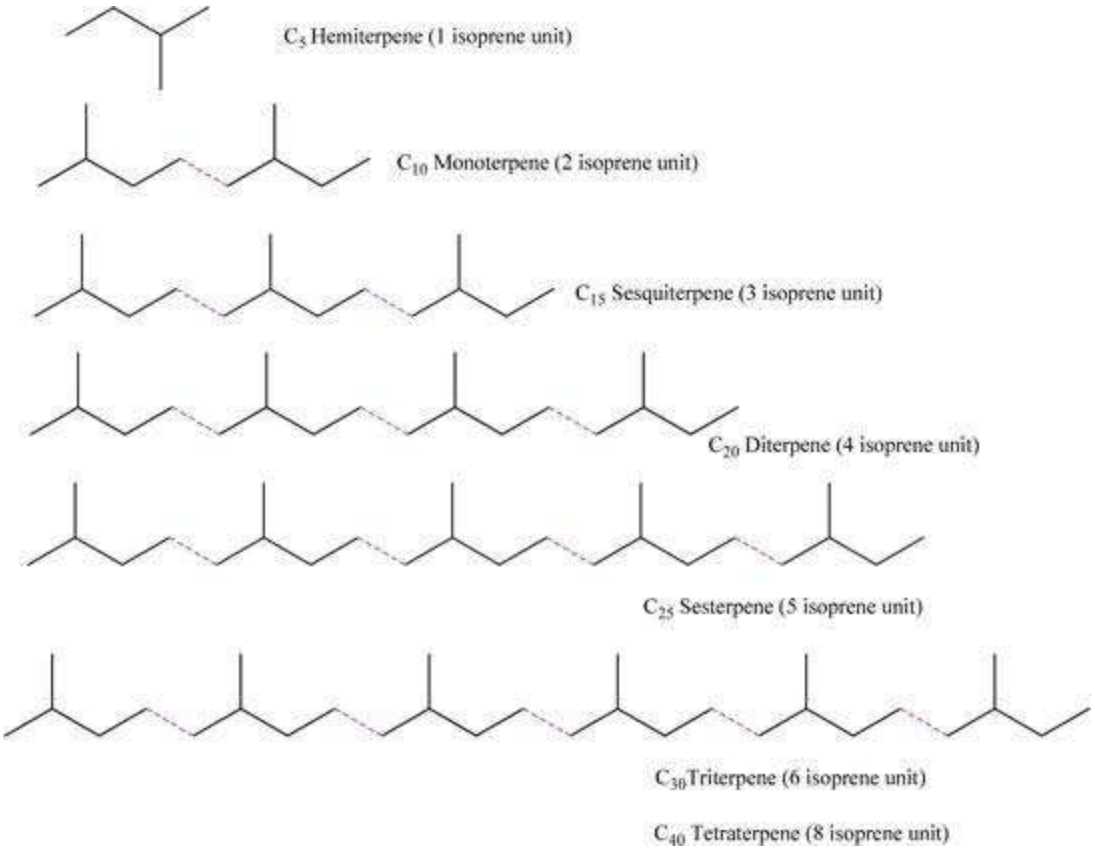

Fig.1.7: Structure of terpenoids.

Terpenoids have medicinal properties such as ant carcinogenic (e.g perilla alcohol), antimalarial (e.g artemisinin), antiulcer, hepaticidal, antimicrobial or diuretic (e.g glycyrrhizin) activity and the sesquiterpenoid antimalarial drug artemisinin and the diterpenoid anticancer drug taxol.

1.5.4 SAPONIN:

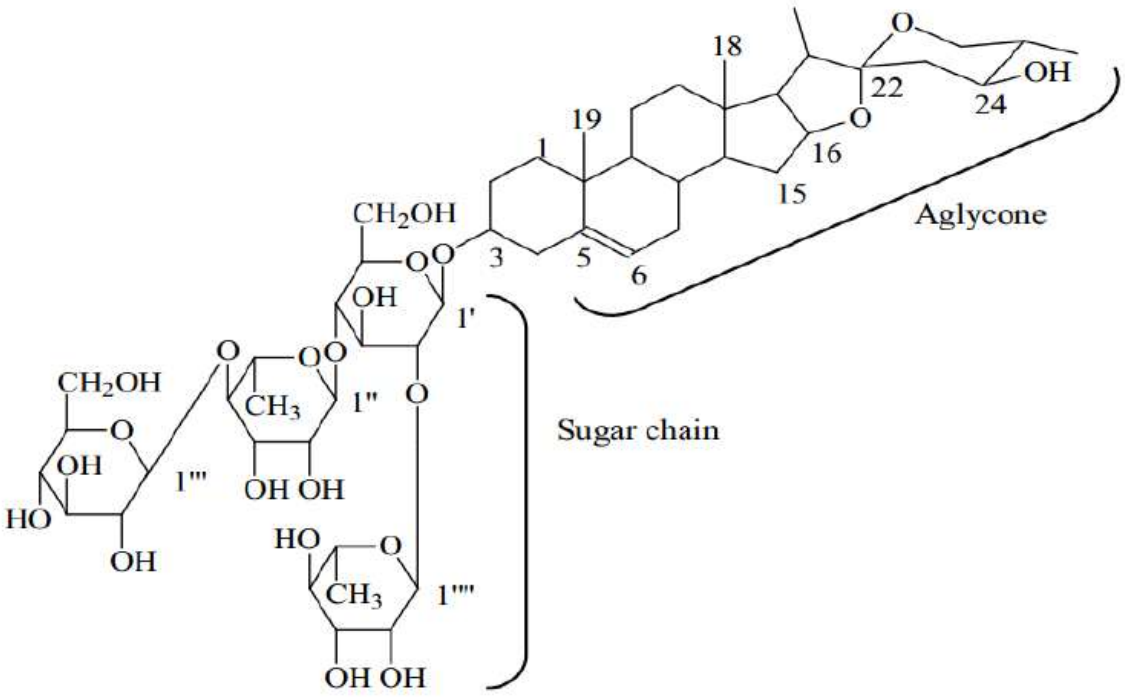

Fig.1.8: Structure of saponin

Saponins are a group of secondary metabolites found widely distributed in the plant kingdom. They are amphipathic glycosides grouped phenomenologically by the soap-like foam they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene or steroid derivative.

Saponins that have one sugar molecule attached at the C-3 positon are called monodesmoside saponins, and those that have a minimum of two sugars, one attached to C-3 and one at C-22 are called bidesmoside saponins.

Many saponins are known to be antimicrobial, to inhibit mould and to protect plants from insect attack. They have also been observed to kill protozoans and molluscs, to be antioxidants, to impair the digestion of protein and the uptake of vitamins and minerals in the gut, to cause hypoocaemia, and to act as antifungal and antiviral (Traore *et al.*, 2000).

1.5.5 POLYPHENOLS:

Polyphenols (Quideau *et al.,* 2011) are a structural class of mainly natural, but also synthetic or semisynthetic, organic chemicals characterised by the presence of large multiples of phenol structural units. Fruits, vegetables, whole grains and other types of foods and beverages such as tea, chocolate and wine are rich sources of polyphenols. Polyphenols are classified by their source of origin, biological function and chemical structure. The majority of polyphenols in plants exist as glycosides with different sugar units and acylated sugars at different positions of the polyphenol skeletons. Polyphenols are classified as

Phenolic acids- they are non-flavonoid polyphenolic compounds which can be further divided into two main types, benzoic acid and cinnamic acid derivatives based on C1-C6 AND C3-C6 backbones.

Flavonoid- they have the C6-C3-C6 general structural backbone in which the two C6 units are of phenolic nature, e.g chalcones, isoflavones etc

Polyphenolic amides- they have the N-containing functional substituents, e/g capsaicinoids, avenanthramides.

High intake of fruits, vegetables and whole grains which are rich in polyphenols have been linked to lower risks of many chronic diseases including cancer, cardiovascular disases, chronic inflammation and many degenerative diseases (Milner, 1994). Polyphenols also induce antioxidant

enzymes such as glutathione peroxidase, catalase and superoxide dismutase respectively and inhibit the expression of enzymes such as xanthine oxidase (Du *et al.*, 2007).

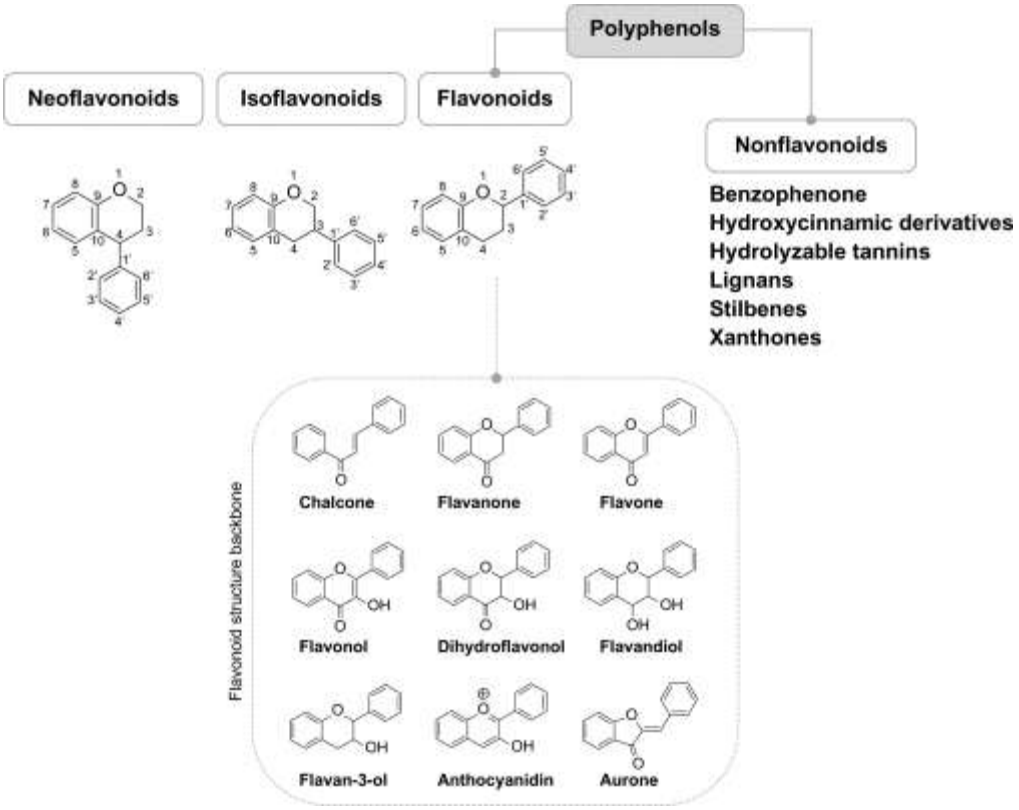

Fig.1.9: structure of polyphenols.

1.5.6 CAROTENOIDS:

Carotenoids are yellow, orange and red organic pigments that are produced by plants and algae, as well as several bacteria and fungi. Carotenoids give the characteristic colour to pumpkins, carrot, corn, tomatoes, canaries, flamingos and daffodils. Carotenoids are found principally in plants, algae and photosynthetic bacteria, where they play a critical role in the photosynthetic process. They also occur in some non-photosynthetic bacteria, yeasts and moulds, where they may carry out a protective function against damage by light and oxygen.

Carotenoids from the diet are stored in the fatty tissues of animals and exclusively carnivorous animals obtain the compounds from animal fat. In the human diet, absorption of carotenoids is improved when consumed with fat in a meal (Mashurabad *et al*., 2017).

Carotenoids are classified according to the structure as follows:

The hydrocarbon carotenoids which are known as carotenes e.g β-carotene.

The oxygenated carotenoids which are derivatives of these hydrocarbons known as xanthophylls e.g zeaxanthin and lutein, spirilloxanthin, echinenone and antheraxanthin.

Carotenoids have the ability to quench singlet oxygen. Because of their roles as antioxidants, carotenoids have been suggested to be protective against coronary vascular disease. Carotenoids also activate the expression of genes which encode the message the message for production of a protein connexin, which is an integral component of the gap junctions required for cell to cell communication. B- Carotene has been shown to protect phagocytic cells from autooxidative damage, enhance T and B lymphocyte proliferative responses, stimulate effector T cell functions, and enhance macrophage, cytotoxic T cell and natural killer cell tumoricidal capacities, as well as increase the production of certain interleukins.

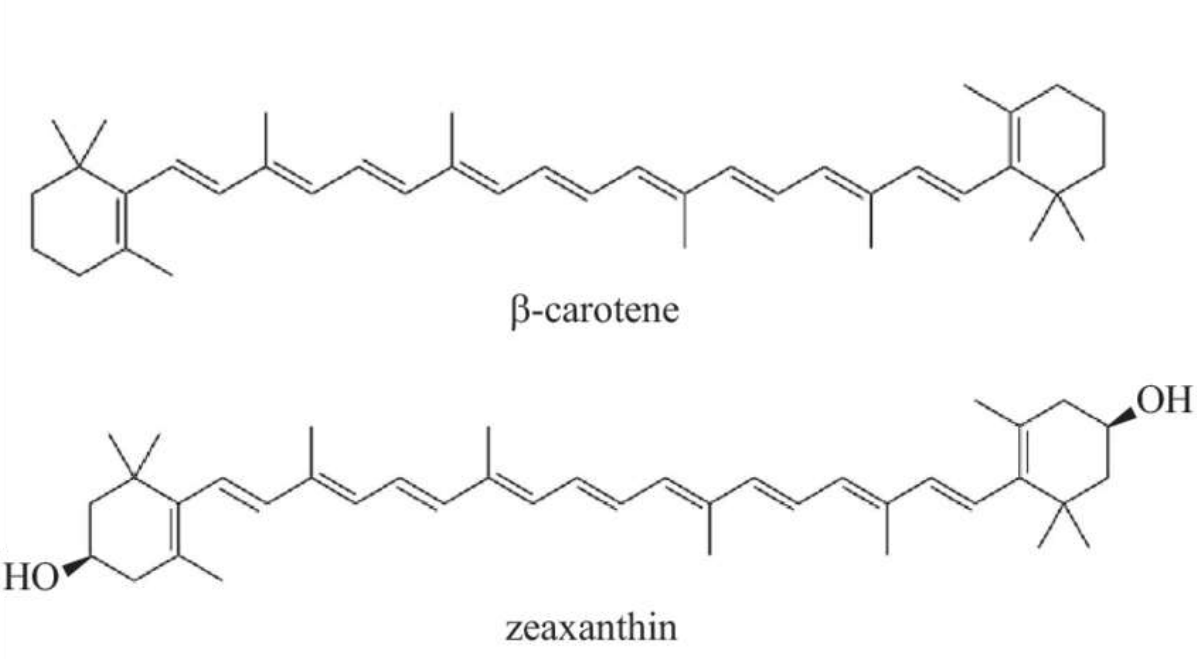

Fig.1.10: Structure of carotenoids.

1.5.2.9 Glycosides:

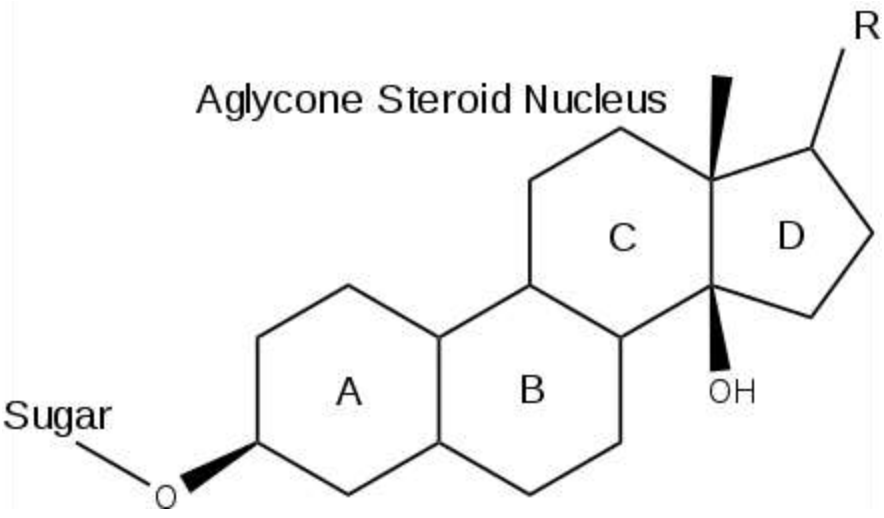

Fig.1.11: structure of glycosides.

A glycoside is a molecule in which a sugar is bound to another functional group via a glycosidic bond. A glycoside is any molecule in which a sugar group is bonded through its anomeric carbon to another group via a glycosidic bond. Glycosides can be lnked by an O-(an 0-glycoside), N-(a glycosylamine), S-(a thioglycoside) or a C-(a C- glycoside) glycosidic bond. Many plants store chemicals in the form of inactive glycosides. These can be activated by enzyme hydrolysis (Brito-Ana and Marlo, 2007) which causes the sugar part to be broken off, making the chemical available for use.

Glycosides are classified as:

- Anthraquinone or anthracene glycosides
- Sterols or cardiac glycosides
- Saponin glycosides
- Cyanogenitic glycosides or cyanophoric glycosides
- Isothiocyanate glycoside
- Flavonoids/flavonol glycoside
- Coumarin and furanocoumarin glycoside
- Aldehyde glycosides
- Phenol glycosides
- Steroidal glycoalkaloids
- Glycosidal bitters or miscellaneous glycosides.

The most important use of cardiac glycosides is its effects in treatment of cardiac failure. Cardiac glycosides also inhibit Na+, K+ ATPase, and consequently increase the force of myocardial contraction. Some cardial glycosides also have antitumor activity.

1.5.7 STEROIDS:

A steroid is a biologically active organic compound with four rings arranged in a specific molecular configuration. Steroids have functions as important components of cell membranes which alter membrane fluidity, and as signalling molecules. Hundreds of steroids are found in plants, animals and fungi.

All steroid molecules are saturated derivatives of phenanthrene, a tri-cyclic aromatic hydrocarbon. The fundamental structure of every steroid molecule also contains a fused cyclopenetane ring. The steroid core structure is typically composed of seventeen carbon atoms, bonded in four fused rings; three six-member cyclohexane ring and one five-member cyclopentane ring.

Animal steroids- include compounds of vertebrate and insect origin e.g ecdysteroids, steroid hormones and cholesterol. Steroid hormones includes sex hormones (e.g androgens, estrogens etc), corticosteroids and anabolic steroids.

Plant steroids- include steroidal alkaloids, cardiac glycosides (Wink *et al*., 2008), the phytosterols and the brassinosteroids.

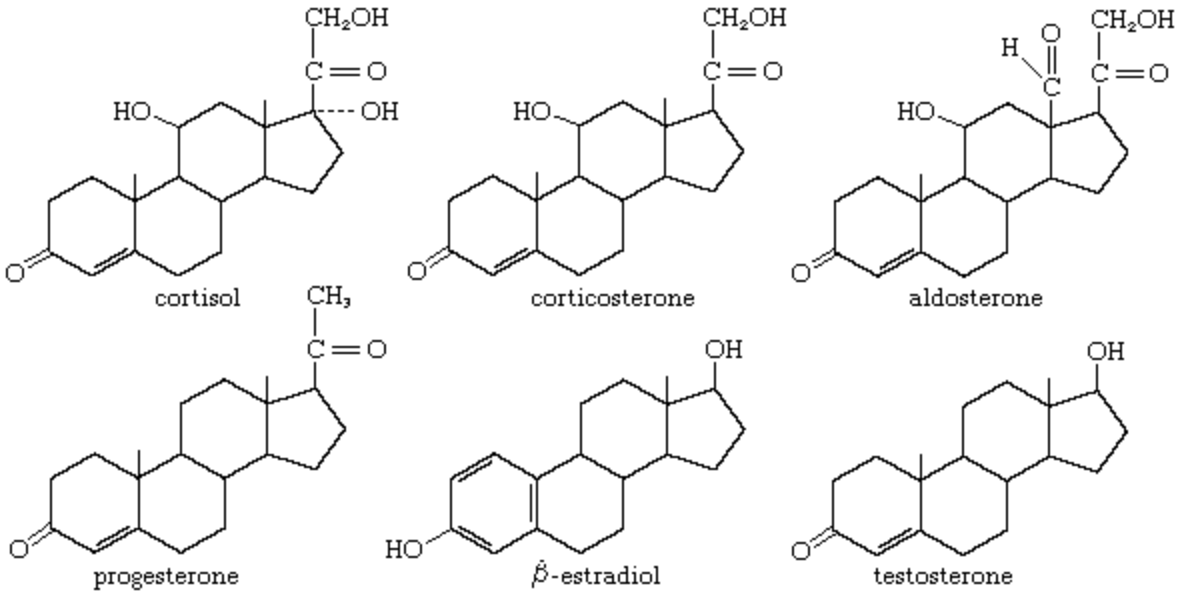

Fig.1.12: Structure of steroids.

CHAPTER TWO

2.0 MATERIALS AND METHODS

The following materials were used during the research study;

- Handkerchief
- Buckets
- *Enantia chlorantha*
- Aluminum foil
- Wister rats
- Cages
- Ceramic plates
- Steel plates
- Glass rod
- Measuring cylinder
- Gloves
- Cotton wool
- Methylated spirit
- Nose mask
- Glucometer and test strip
- EDTA container
- Lithium heparin container
- Plain container
- Masking tape
- Universal bottle

- Test tubes
- Test tubes racks
- Dissecting set
- Micro-centrifuge tubes
- Lancet
- 250ml and 1000ml beakers
- Blade and scissors
- Syringe (2ml and 5ml)
- Insulin syringe
- pH meter
- Pipette
- Separating funnel
- Retort stand
- Glass jar
- Digital weighing scale
- Sensitive weighing balance
- Mortar and pestle
- Spatula
- Oral gavage
- Spectrophotometer
- Centrifuge
- Cuvette
- Micropipette

2.1 CHEMICALS AND REAGENT USED

The following chemicals and reagent were used during the research study;

- Ethyl-Acetate
- Sodium citrate
- Citric acid
- Ethanol
- Potassium hydroxide
- Distilled water
- Picric acid
- Chloroform
- Cholesterol reagent kit
- Triglycerides reagent kit
- Total protein reagent kit
- HDl-Cholesterol reagent kit

2.2 METHODOLOGY

All the basic methods applied in the course of the research are as follows,

2.2.1 PREPARATION OF PLANT EXTRACTS

The pulverized extract was soaked in hydroethanol with a ratio of ethanol to distilled water 80:20 (800ml of Ethanol and 200ml of distilled water) in a jar. The mixture was stirred using a glass rod until it was free. It was then allowed to stand for three days after three days; the crude extract was separated from the crude extract by sieving using a white unused handkerchief. The crude extract gotten were poured into clean jars and labeled. The crude extract was again soaked in hydroethanol

with the same ratio of ethanol to distilled water, 80:20 in a jar for another three days. This process is termed exhaustive extraction. The crude extract were then sent to pharmaceutical chemistry staff research laboratory for freeze drying in a time frame of 24hours.

2.2.2 PREPARATION OF THE ANIMALS USED

Male Wistar rats were purchased of age (16weeks), kept in clean and serene cages and left to acclimatize for two weeks by feeding them *ad lebitum* with normal poultry feed called grower mash. After acclimatization, the Wister rats were divided into 7 groups with six animals in each group and kept in separate cage. The animals were then weighed on a weighing balance and each rat was stained on various body parts such as their tails, head and back using picric acid. This was for identification purposes.

2.2.3 DIET FORMULATION

Both High fat diet and Normal diet (HFD &ND respectively) were prepared. The content of the high fat diet includes; corn starch from a carbohydrate source, fish meal from a protein source, soybean oil from fat and oils, butter from fat and oil, sucrose from a simple sugar, cellulose gotten from a fiber source, vitamin mix from vitamins and mineral mix from minerals. The normal diet involved all the contents of the high fat diet with an exception of butter. The cornstarch was dried for three days consecutively, before the formulation of feed, after the drying process the content of the High fat diet and Normal diet were measured, using this measurement, these contents were mixed and pelleted it was left to dry for a few hours. The feeds were weighed and 100mg of high fat diet and normal diet were administered to various groups of animals,

Table 2.1: Formular for normal diet content

RAW MAERIALS	SOURCE	WEIGHT (grams)
Cornstarch	Carbohydrate	2,562.5
Cellulose	Fiber	875
Sucrose	Simple sugar	625
Fish meal	Protein	312.5
Soybean oil	Fat and oil	250
Mineral mix	Minerals	62.5
Vitamin mix	Vitamins	218.75

Table 2.2: Formular for high fat diet content

RAW MATERIALS	SOURCE	WEIGHT (grams)
Corn starch	Carbohydrate	5,125
Cellulose	Fiber	625
Sucrose	Simple sugar	1,250
Fish meal	Protein	1,750
Soybean oil	Fats and oil	500
Mineral mix	Mineral	437.5
Vitamin mix	Vitamin	125
Butter	Fats and oil	2,687.5

2.2.4 FRACTIONATION USING ETHYLACETATE AND HYDROETHANOL

This was done using a separation technique and the principle behind this fractionation is solvent to solvent partition it was carried out via a separating funnel. The marc gotten after freeze drying was weighed and mixed with distilled water, stirred to dissolve and ethanol was added to aid complete dissolving. The ratio of ethanol to water was 4:1. The marc was poured into the separating funnel and ethyl acetate was added to it to get the ethyl acetate fraction. The ethyl acetate collects polar component from it and layer of ethyl acetate fraction was formed above and was collected by separating the marc which drains out through the valve leaving behind the less dense acetyl acetate fraction. This process was repeated at an interval of 15 minutes until there was a colorless layer formed indicating that the ethyl acetate had completely taken the polar components from the marc. The marc was poured back into the separating funnel and hydro-ethanol was added to it to collect the remaining constituents in the marc. The Ethyl acetate fraction and hydro-ethanol fraction were then sent to pharmaceutical chemistry staff research laboratory for freeze drying. The freeze dried fractions were sent to a lab for phytochemistry analysis.

2.2.5 EXPERIMENTAL DESIGN

The male wistar rats were arranged into seven groups which are

- **Group One:-** This is the normal control, they were feed with normal diet(100mg) and water only for nine weeks throughout the duration of the study. Diabetes mellitus was not induced and no treatment of any form was administered.

- **Group Two:-** This is the diabetic control, they were feed with high fat diet (100mg)) and water only for nine weeks. Diabetes mellitus was induced using streptozotocin and no treatment of any form was administered throughout the duration of the study.

- **Group Three:-** This is the positive control, they were feed with high fat diet (100mg)) and water only for nine weeks. Diabetes mellitus was induced using streptozotocin and standard diabetic mellitus drug called metformin (500g) administered orally, throughout the duration of the study.
- **Group Four:-** They were feed with high fat diet (100mg)) and water only for nine weeks. Diabetes mellitus was induced using streptozotocin and Crude hydroethanol extract (200mg) was administered orally daily, throughout the duration of the study.

- **Group Five:-** They were feed with high fat diet (100mg)) and water only for nine weeks. Diabetes mellitus was induced using streptozotocin and Crude hydroethanol extract (400mg) was administered orally, daily throughout the duration of the study.
- **Group Six:-** They were feed with high fat diet (100mg)) and water only for nine weeks. Diabetes mellitus was induced using streptozotocin and Ethyl acetate extract (200mg) was administered orally, daily throughout the duration of the study.

- **Group Seven:-** They were feed with high fat diet (100mg)) and water only for nine weeks. Diabetes mellitus was induced using streptozotocin and Ethyl acetate extract (400mg) was administered orally, daily throughout the duration of the study.

2.3.0 INDUCING OF DIABETES MELLITUS USING STREPTOZOTOCIN

The animals were feed for nine weeks and their weight was being monitored weekly until they were obsessed**.** The streptozotocin drug was administered through intra-peritoneal route using an insulin syringe and the fasting blood glucose was checked after three days by collecting blood

samples from the tail tip of the wistar rat. And a rat was said to be diabetic until the fasting blood glucose was above 200mg/dl.

2.3.1 PREPARATION OF STREPTOZOTOCIN (STZ) TO BE ADMINSTERED

0.03 was multiplied against the weight of each wistar rat the cumulative figure was summed up and the amount of STZ weighed was in correlation to that figure. The STZ was weighed using a sensitive weight balance and it was dissolved into 150ml of cold citrate buffer with a pH of 4.5

The STZ drug is light sensitive needs to be cold; the beaker that houses the STZ is always wrapped inside a foil paper to prevent light from contacting the STZ.

Then the volume of STZ to be administered was calculated using;

$$\text{Volume of STZ} = \frac{(0.03 \times \text{weight of wistar rat}) \times 2.0\text{mls}}{\text{Summed up Cumulative figure}}$$

2.3.2 PREPARATION OF COLD CITRATE BUFFER

2.989g of sodium citrate and 2.1g of citric acid was weighed. It was then dissolved with 50ml of cold distilled water and transferred into a measuring cylinder, where it was topped up to 100ml with cold distilled water. The pH was taken at 4.5. The solution was slightly acidic to allow the streptozotocin carry out its destructive effects on the pancreas of the animal leading to diabetes mellitus. Due to environmental factor the pH reduced and was corrected using a base potassium hydroxide.

2.3.3 FASTING BLOOD SUGAR

The wistar rats were starved overnight and blood was obtained from the tail tip. Methylated spirit was used to clean the tail tip and a lancet was used to prick the tail tip, then two drops of blood was placed on the glucose test strip and inserted into the glucometer device and within 15 seconds the value for the blood glucose level was displayed on the LED screen using in mg/dl. All of this is usually done before 10:00am in the morning. This was done usually every three days immediately after diabetes mellitus was induced.

2.5.0 EUTHANIZATION AND SAMPLE COLLECTION FROM WISTAR RATS

After 14days of administration of standard drug (metformin), N-hexane extracts and Ethyl acetate extracts, the wistar rats were euthanized then blood samples and organs samples were harvested from each animal. The fasting blood glucose was taken one last time and the rats were placed into a container with cotton balls soaked with chloroform and after few minutes the rats would lose consciousness. Immediately the rats are inverted and a section of the peritoneum cavity is cut open and extended towards the thoracic cavity of the wistar rat using a dissecting blade. Blood was collected using a 5ml syringe by piercing the left ventricle of the heart and blood was drawn and placed into EDTA container, lithium heparin containers and micro-centrifuge tubes and labeled boldly accordingly to the label of each animal.

Also organs like liver, pancreas and muscle samples were collected and preserved with formalin inside test tubes and kept in a freezer. The duration of feeding, inducing diabetes and treatment of the wistar rats was 11 weeks.

CHAPTER THREE

3.0 RESULTS

ANTIDIABETIC EFFECTS OF *ENATIA CHLORANTHA* (EC) STEM BARK EXTRACT IN STREPTOZOTOCI N-INDUCED DIABETIC RAT

The antidiabetic activity of extracts of EC stem bark in type 2 diabetes model is shown in Table 1. The rats induced with type 2 diabetes showed significant increase ($p>0.05$) of the blood glucose level, when compared to the normal control group on day three. Also there was a dose-dependent reduction in blood glucose levels in the diabetic treated (200mg/kg and 400 mg/kg body weight) rats on days 7 and 14 when compared to diabetic control. Similarly administration of the standard drug, metformin significantly lower the increased glucose levels progressively throught the experiment.

Table 1. Antidiabetic effects of *Enatia chlorantha* stem bark extract

Group	Fasting Blood Glucose Level (mg/dl)			
	Day 0	Day 3	Day 7	Day 14
Group I (Normal control)	80.00 ± 2.06^{a}	71.00 ± 2.66^{a}	97.00 ± 2.06^{a}	85.62 ± 3.09^{a}
Group II (35mg/kgbw STZ)	$^{a}88.00 \pm 1.19$*	$^{b}297.50 \pm 1.46$*	$^{c}449.00 \pm 7.04$*	$^{c}454.00 \pm 7.80$*

Group III (Metformin)	[a]79.50 ± 0.84	[b]357.00 ± 4.90**	[c]103.50 ± 1.46**	[d]84.5.80 ±1.46**
Group IV (200mg/kgbw H.E)	[a]80.00 ± 1.19	[b]383.00 ± 3.36**	[c]223.00 ± 2.06**	[d]195.00 ±3.76**
Group V (400mg/kgbw H.E)	[a]78.00±1.18	[b]313.50±1.46**	[c]289.5±6.00**	[d]177.00±3.14**
Group VI (200mg/kgbw E.A)	[a]80.5±1.46	[b]317.5±0.84**	[c]366.50±0.84**	[d]390.5±4.03**
Group VII (400mg/kgbw E.A)	[a]82.50±1.85	[b]351.50±0.84**	[c]173.00±4.44**	[d]81.5±2.22**

Values are expressed as mean ± SEM (n=7); values with different alphabet letters are significantly different ($p < 0.05$) from one another. *mean is significant ($P \square 0.05$) when compared with the control; ** mean is significant ($P \square 0.05$) when compared with diabetic control group.

Group1: Normal Control; Group II (Diabetic Control): high fat diet for 6 weeks and low dose of STZ (35 mg/kg) only for ; Group III; high fat diet for 6 weeks and low dose of STZ + Metformin (200 mg/kg bw) Group IV; high fat diet for 6 weeks and low dose of STZ + 200 mg/kg bw of hydroethanol extract of EC stem bark Group V; high fat diet for 6 weeks and low dose of STZ + 400 mg/kg bw of hydroethanol extract

of EC stem bark; Group VI; high fat diet for 6 weeks and low dose of STZ + 200 mg/kg bw of ethyl acetate fraction of EC stem bark Group VII: 400 mg/kg bw of ethyl acetate fraction of EC stem bark

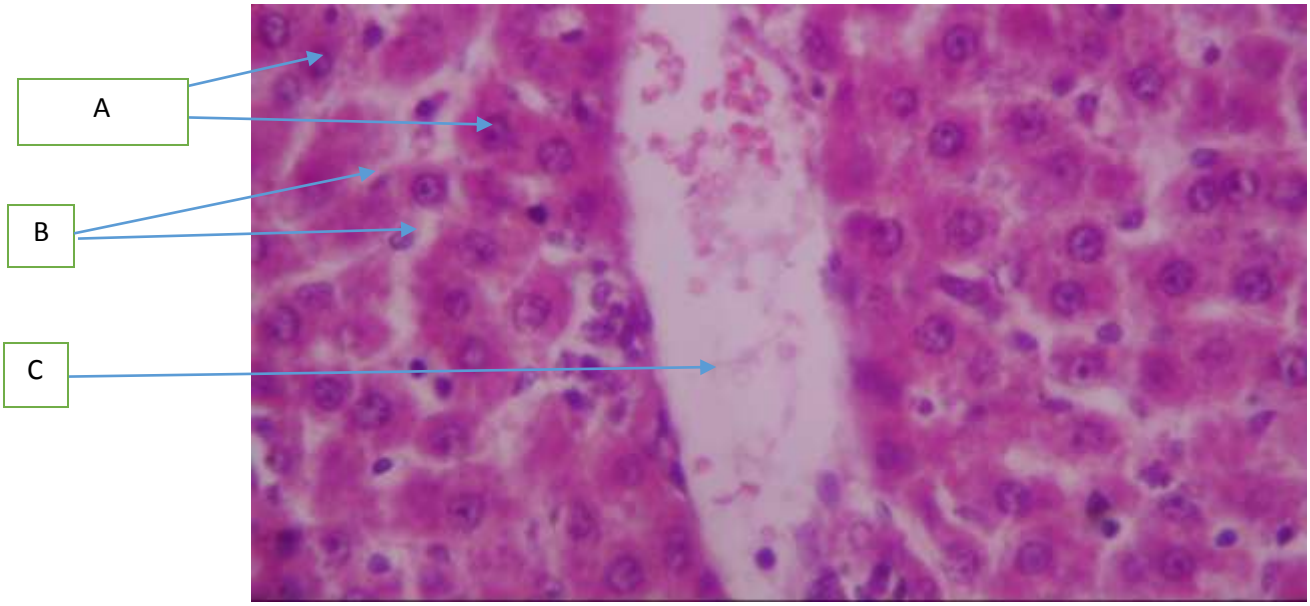

Plate 1. Control. Composed of: A, hepatocytes, B, sinusoids and C, central vein (H&E x 400)

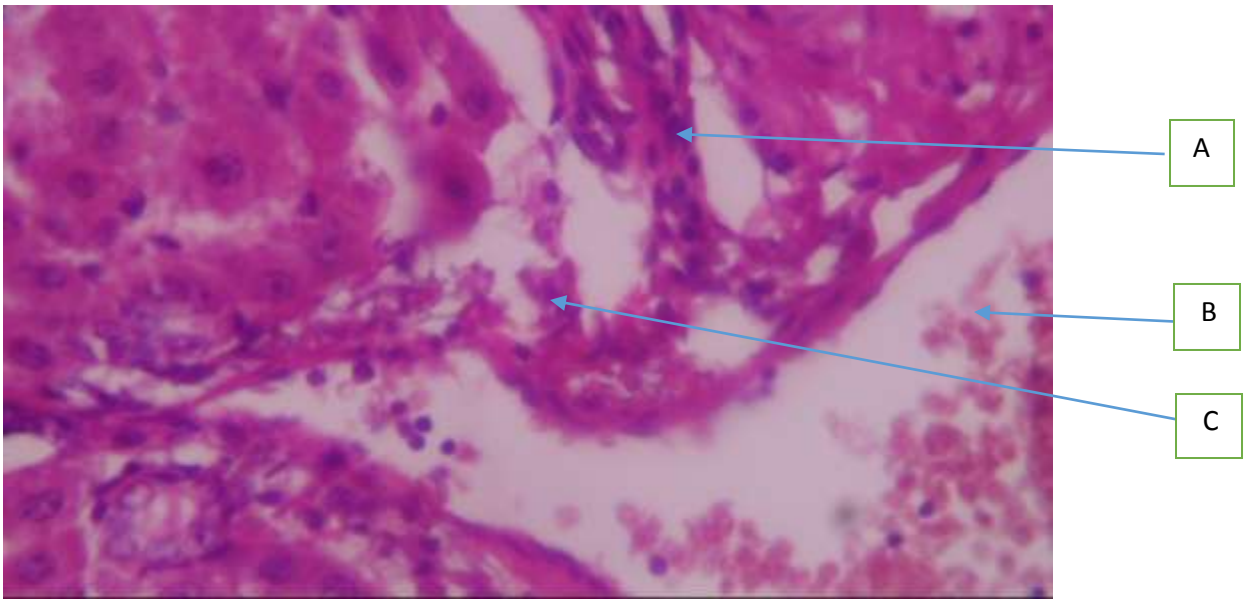

Plate 2. Rat given Streptozotocin (STZ) only showing: A, periportal infiltrates of inflammatory cells, B, portal congestion and C, zonal necrosis (H&E x 400)

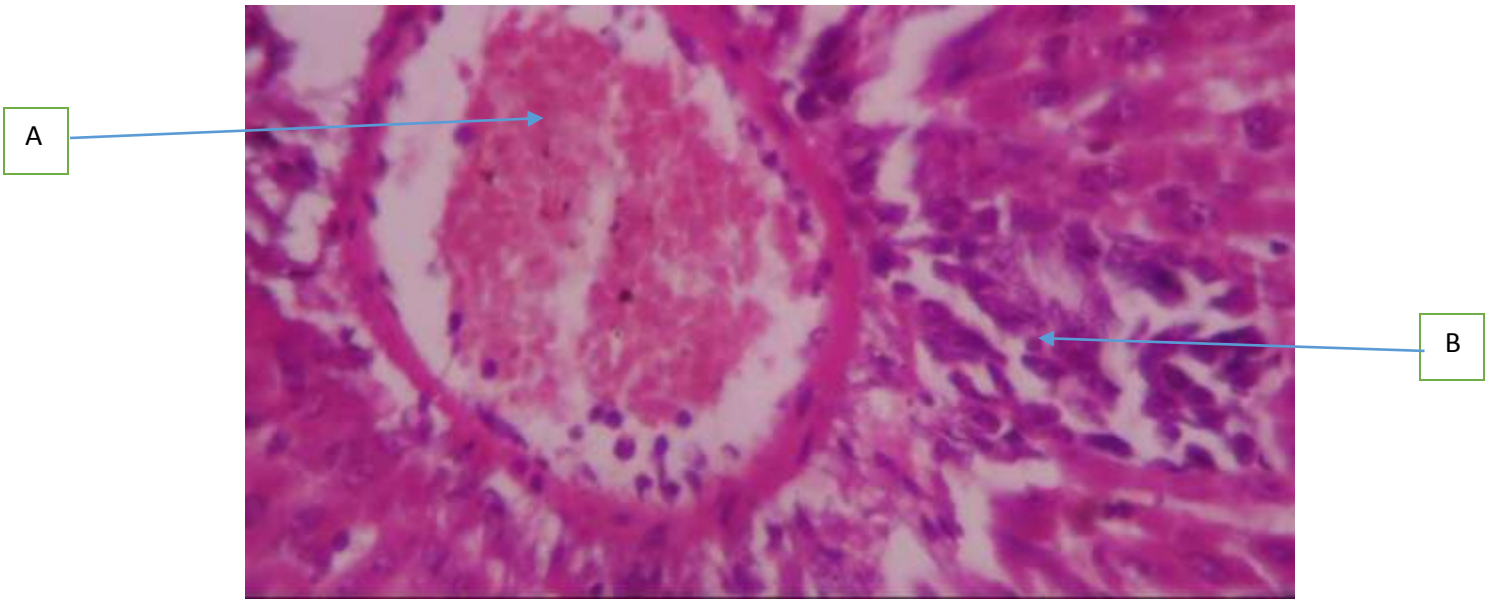

Plate 3. Rat given Streptozotocin (STZ) + Metformin showing: A, portal congestion and B, focal periportal infiltrates of inflammatory cells (H&E x 400)

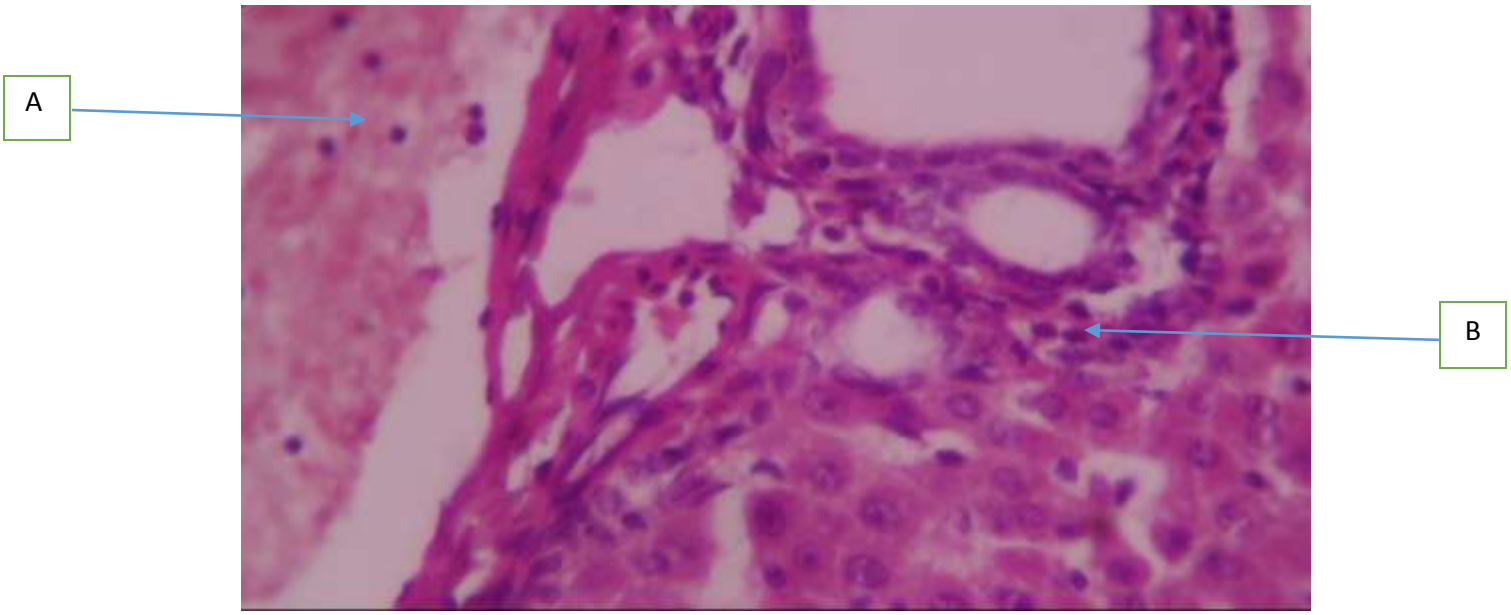

Plate 4. Rat given Streptozotocin (STZ) + 200mg Crude Extract showing: A, portal congestion and B, mild periportal infiltrates of inflammatory cells (H&E x 400)

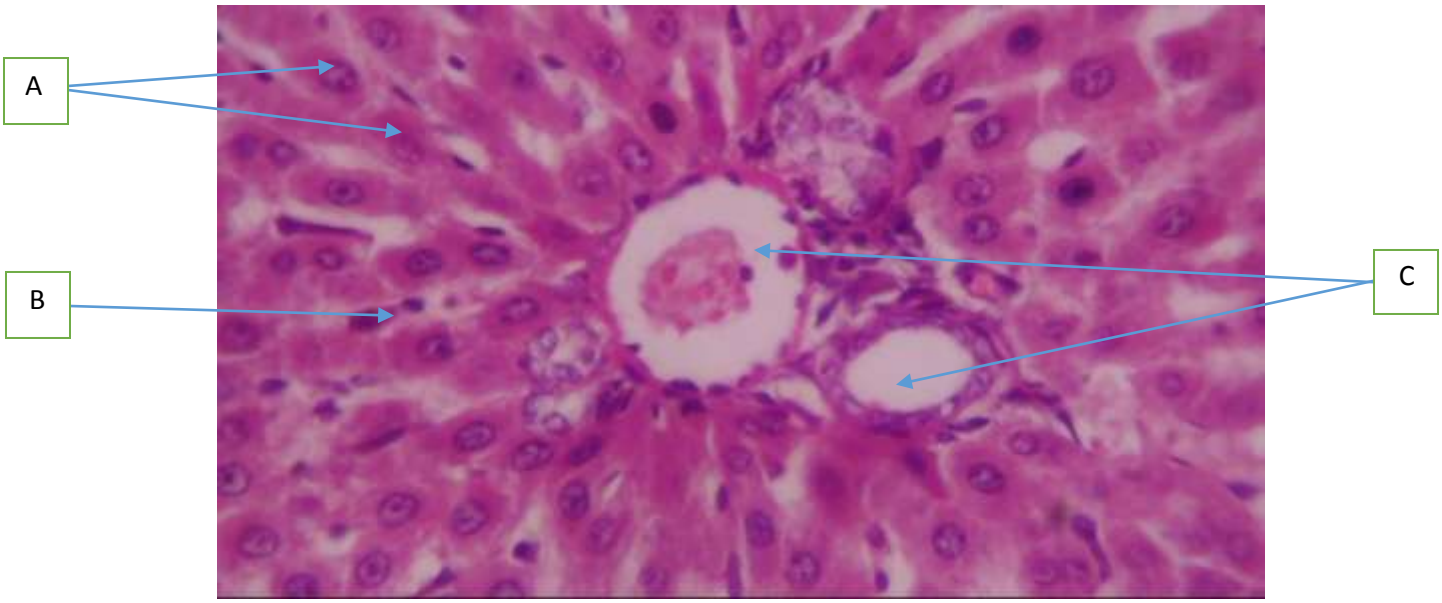

Plate 5. Rat given Streptozotocin (STZ) + 400mg Crude Extract showing: A, normal hepatocytes, B, kupffer cell activation and C, normal portal triad

(H&E x 400)

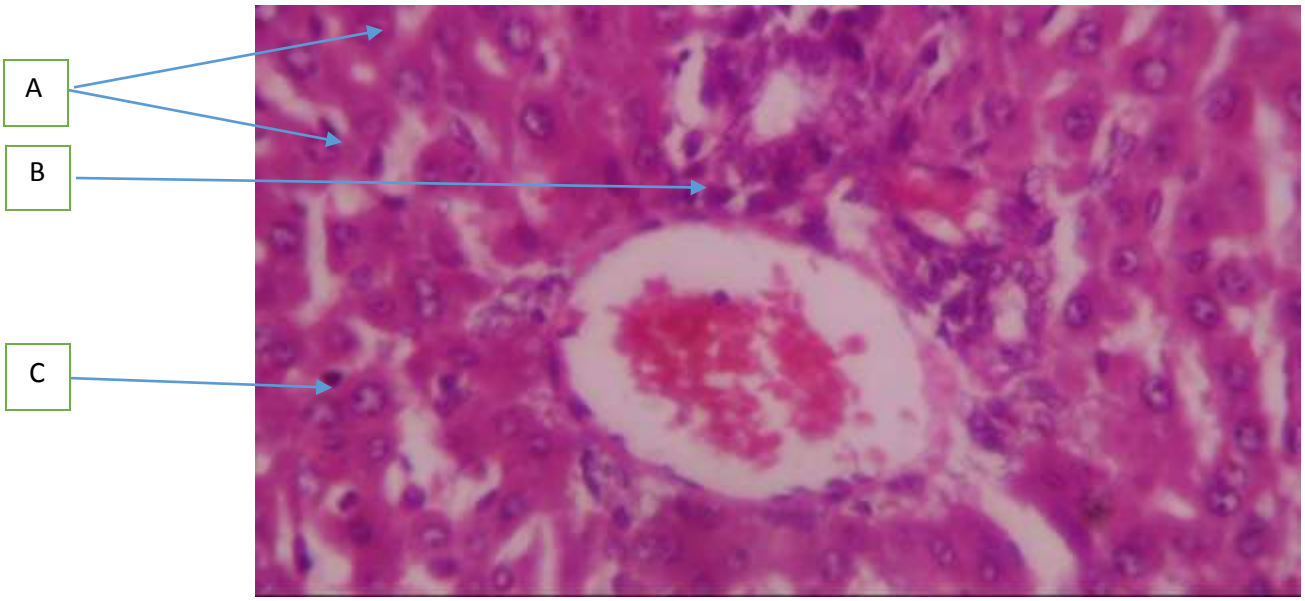

Plate 6. Rat given Streptozotocin (STZ) + 200mg Ethylacetate Extract showing: A, normal hepatocytes, B, focal periportal infiltrates of inflammatory cells and C, kupffer cell activation (H&E x 400)

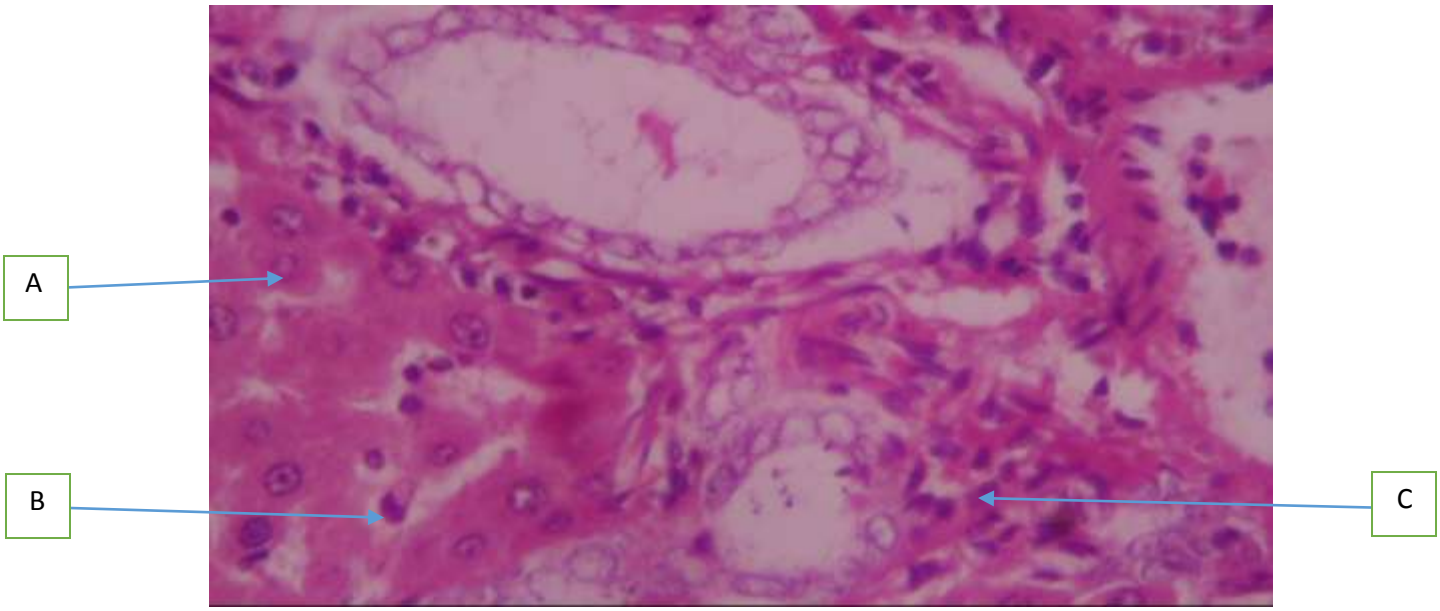

Plate 7. Rat given Streptozotocin (STZ) + 400mg Ethylacetate Extract showing: A, normal hepatocytes, B, kupffer cell activation and C, mild periportal infiltrates of inflammatory cells (H&E x 400)

CHAPTER 4

DISCUSSION AND CONCLUSION

Streptozotocin is widely used in experiments to induce a diabetic model due to its ability to cause β-cell toxicity in mammals. The significant indices in the pathogenesis of diabetes are pancreatic dysfunction and cell death (Kumar N, Kar A 2015). STZ induces diabetes by creating reactive oxygen species and decreasing antioxidant capacity, hence causing cytotoxicity in pancreatic beta cells. A concoction of Enantia Chlorantha has been used to treat malaria symptoms, aches, wounds, boils, vomiting, yellow bitter, fever, chills, sore, spleen in children (Tsabang et al., 2012). The stem bark decoction, taken orally, has also been reported to treat intestinal worms, intestinal spasms, malaria and sexual asthenia. The same decoction is effective against hepatitis, jaundice, urinary tract infections and typhoid fever.

According to Abd El-Kader Am *et al.,* 2019, liver and kidney are represented as major target organs for drugs. In the current work, in addition to pancreatic damage, STZ administration led to hepatic damage causing hepatic malfunction and alterations in the circulating concentrations of hepatic damage enzymes. Monitoring the levels of serum ALT and AST, which are also sensitive serological indicators of hepatotoxicity, are indicative of a clinical diagnosis of the disease and damage to the structural integrity of liver. Oxidative stress related to STZ-induced diabetes has been found in response to higher levels of these enzymes in serum, as also found in our study, confirming that STZ administration induced liver tissue damage and significant elevation in serum ALT and AST levels. Again, Aloe Vera administration to animals treated with STZ reduced these two marker enzymes, which was also reinforced by histological examination and revealed a significant improvement in liver histological damage in the STZ-treated group. Similar effects of various other medicinal plants have been observed.

The liver contain endocrine tissues. The healthy liver looks like an arranged cells with a vein. The round circle looking like an eye or iris is the nucleus of the liver cell while the purple like or pink like color are the cytoplasm of the hepatocytes. The normal shape of the hepatocytes is strap while the older hepatocytes have two nuclei and are separated by sinusoids-hepatic sinusoids are modified capillaries (appear to be whitish spaces in between the cytoplasm of the hepatocytes). Sinusoids are found in endocrine tissues. They are modified capillaries because their basal Lamina is fenestrated

In the liver cell is the central vein-the structural center of the hepatic Lubiole.

The control group fed with normal food and wasn't induced with Streptozotocin have the hepatocytes and central vein organized, orderly and patterns are regular.

When the rats were induced with Streptozotocin (negative control), the portal vein became narrow. Vasculoparthy (sickness of the red blood cells), Periportal infiltrates of the inflammatory cells (periportal hepatitis), portal congestion, zonal necrosis, Empty spaces, Inflammation of the hepatocytes around the portal zone of the liver

When Metformin (a standard diabetic drug) was used, the blood vessels were not affected. There was still portal hepatitis, necrosis and inflammation of the hepatocytes. It was better than the negative control.

200mg of the crude extract was administered, necrosis was reduced and inflammation persisted but mild. It did a better job than the Metformin

400mg of the crude extract ameliorated the disease, no inflammation, no congestion, no necrosis, fine hepatocytes, hepatocytes radiating like rays. Bile ducts are seen too. Sinosoids are seen

properly and Kupffer cells were observed to be activated. Kupffer cells are like macrophages and help with immune defenses.

200mg ethyl acetate tried in ameliorating the effects of the Streptozotocin damage. Small inflammation was observed, small congestion was observed, necrosis were really reduced, there was Kupffer cell activation too and did a better job than Metformin

400mg ethyl acetate and the inflammation became more obvious compared to the 200mg of ethyl acetate which did a better job at ameliorating the inflammation. The bile ducts were obvious, no congestion, mild inflammation.

Conclusion

The crude extract at 400mg did a better ameliorating job in the liver than the 200mg crude extract, the 200 mg ethyl acetate, the 400mg ethyl acetate and the Metformin.

REFERENCES

Abd El-Kader Am, Awadalla Ea, Gabr Sa, Nour Ah (2019). Evaluation of the Histopathological and Biochemical Effect of Aloe Vera Aqueous Extract on Diabetes Mellitus Induced By Streptozotocin in Rats. *Asian Journal of Pharmaceutical and Clinical Research*. 12:6; 38-45

American Diabetes Association (2010) Diagnosis and classification of diabetes mellitus. *Diabetes Care* **33** Suppl 1: S62-69.

American Diabetes Association. (2014). Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care Volume* **37,** Supplement 1,

Amos AF, McCarty DJ, Zimmet P: The rising global burden of diabetes and its complications: estimates and projections to the year 2010. Diabet Med 14 (Suppl. 5): S1–S85, 1997

Ayoka, A.O., Akomolafe, R.O., Akinsomisoye, O.S and Ukponmwan, O.E. (2008). Medicinal and economic value of Spondias mombin. *Afr. J. Biomed. Res.* **11;** 129-136.

Bendinelli, B (2012). Association between dietary meat consumption and incident type 2 diabetes: the EPIC-InterAct study. *Diabetologia* **56:** 47-59

DIAMOND Project Group (2006). Incidence and trends of childhood type 1 diabetes worldwide *Diabet Med* **23**: 857 - 66.

Dolan (1997). ME: Inhibition of DNA repair as a means of increasing the antitumor activity of DNA active agents. *Adv Drug Del Rev* **26:** 105–118

Gale, E.A, and Gillespie, K.M. (2001). Diabetes and gender. *Diabetologia* **44:** 3-15

Holt G. I. (2004). Diagnosis, epidemiology and pathogenesis of diabetes mellitus an update for Psychiatrists. *Br. J. Psychiatry*. **184:** s55- s63.

Hypponen, E., Laara, E., Reunanen, A., Jarvelin, M.R, and Virtanen, S.M. (2001) Intake of vitamin D and risk of type 1 diabetes: a birth-cohort study. *Lancet* **358:** 1500-3.

Iwu, M.M. (1993). Handbook of African Medical Plants. *New York: CRC Press* p. 33–5

Jiofack, T., Ayissi, I., Fokunang, C., Guedje, N., and Kemeuze V. (2009). Ethnobotany and phytomedicine of the upper Nyong valley forest in Cameroon. *African Journal of Pharmacy and Pharmacology* **3**(4):144–50.

Joy, S.M., Little, E., Maruthur, N.M., Purnell, T.S., and Bridges, J.F (2013) Patient preferences for the treatment of type 2 diabetes: a scoping review. *Pharmacoeconomics* **31**: 877-892.

Kayode, J. (2006). Conservation of indigenous medicinal botanicals in Ekiti State, Nigeria. *J Zhejiang Univ Sci B* **7** (9):713–8.

Kumar N, Kar A. (2015). Pyrroloquinoline quinone (PQQ) has potential to ameliorate streptozotocin-induced diabetes mellitus and oxidative stress in mice: A histopathological and biochemical study. Chem Biol Interact 2015. 240:278-90.

Langenberg, C., Sharp, S.J, Franks, P.W, et al. (2014) Gene-lifestyle interaction and type 2 diabetes: the EPIC interact case-cohort study. *PLoS Med* **11**. pp100-1647

Olivier, T.T., Francis, N. T., and Armel, J. S. (2016).Traditional uses, phytochemical and pharmacological profiles, and toxicity of Enantia chlorantha (Oliver): An overview. *Edorium J Med* **3**:12–18.

Raju, S.M, and Raju B (2010). Illustrated medical biochemistry. 2nd Edition. *Jaypee Brothers Medical Publishers ltd, New Delhi, India*. 645pp

Ronald, G, and Zubin, P. (2013). Definition, Classification and Diagnosis of Diabetes, Prediabetes and Metabolic Syndrome. *Canadian Journal of Diabetes*. **37**. S8-s11

Samreen, R. (2009). Diabetes mellitus. *Scientific Research and Essay* Vol. **4** (5) pp. 367-373

Sarah, W., Gojka, R., Anders, G., Richard, S., and Hilary K (2004). Global Prevalence of Diabete. *Diabetes Care* **27**:1047–1053.

Sicree, R., Shaw, J and Zimmet P. (2006). The Global Burden. Diabetes and Impaired Glucose Tolerance. Prevalence and Projections. *In: Gan, D. ed. Diabetes Atlas, 3rd edn. Brussels: International Diabetes Federation,* pp. 16–103.

Taylor, J.L.S., Rabe, T., Mcgaw, L.J., Lager, A.k., and Van Staden, J. (2001). Toward the scientific validation of traditional medicinal plants. Plant Growth Regul. 34:23-37.

Tene, T., Francis, N. T., and Armel, J. S. (2016). An overview Olivier: Traditional uses, phytochemical and pharmacological profiles, and toxicity of Enantia chlorantha (Oliver): *Edorium J Med* **3**:12–18

Tobias, D.K., Hu, F.B., Chavarro, J., Rosner, B., Mozaffarian, D., and Zhang, C. (2012). Healthful dietary patterns and type 2 diabetes mellitus risk among women with a history of gestational diabetes mellitus. *Arch Intern Med* **172**:1566–1572

Tsabang, N., Fokou, P.V., Tchokouaha, L.R. (2012). Ethnopharmacological survey of Annonaceae medicinal plants used to treat malaria in four areas of Cameroon. *J Ethnopharmacol* **139**(1):171– 80.

Zimmet, P., Alberti, K.G. and Shaw, J. (2001). Global and societal implications of the diabetes epidemic. *Nature*. **414**:782–787

Appendix

PREPARATION OF STANDARD TREATMENT DRUG TO BE ADMINISTERED

Two tablets each of metformin (500mg) was crushed to powder using a mortar and pestle then was dissolved into 10ml of distilled water and stirred until it dissolved completely. The volume to be administered depends on the weight of the wistar rat.

PREPARATION OF CRUDE HYDROETHANOL EXTRACTS TO BE ADMINISTERED

- **For The 200mg Dosage Of Crude Hydroethanol Extract**

After crude hydroethanol extract was freeze dried 6.0g was dissolved into 60mls of distilled water inside a beaker and it was stirred continuously to ensure complete dissolution.

To get amount of crude hydroethanol extract in (mg)

$$\text{Crude hydroethanol extract in (mg)} = \frac{200\text{mg} \times \text{Body weight of animal (g)}}{1000\text{g}}$$

To get volume of crude extract to be administered per body weight (g) of animal

$$\text{Volume of crude extract (ml)} = \frac{60\text{ml} \times \text{Crude hydroethanol extract in (mg)}}{6000\text{mg}}$$

- **For The 400mg Dosage Of Crude Hydroethanol Extract**

To get amount of crude hydroethanol extract in (mg)

$$\text{Crude hydroethanol extract in (mg)} = \frac{400\text{mg} \times \text{Body weight of animal}}{1000\text{g}}$$

To get volume of crude extract to be administered per body weight (g) of animal

$$\text{Volume of crude extract (ml)} = \frac{60\text{mls} \times \text{Crude hydroethanol extract in (mg)}}{6000\text{mg}}$$

PREPARATION OF ETHYL ACETATE EXTRACTS TO BE ADMINISTERED

- **For The 200mg Dosage Of Ethyl Acetate Extracts**

After ethyl acetate extracts was freeze dried 6.0g was dissolved into 60mls of distilled water inside a beaker and it was stirred continuously to ensure complete dissolution.

To get amount of ethyl acetate extract in (mg)

$$\text{Ethyl acetate extract in (mg)} = \frac{200\text{mg} \times \text{Body weight of animal (g)}}{1000\text{g}}$$

To get volume of ethyl acetate extract to be administered per body weight (g) of animal

$$\text{Volume of ethyl acetate extract (ml)} = \frac{60\text{ml} \times \text{Ethyl acetate extract in (mg)}}{6000\text{mg}}$$

- **For The 400mg Dosage Of Ethyl Acetate Extracts**

To get amount of ethyl acetate extract in (mg)

$$\text{Ethyl acetate extract in (mg)} = \frac{400\text{mg} \times \text{Body weight of animal}}{1000\text{g}}$$

To get volume of ethyl acetate extract to be administered per body weight (g) of animal

$$\text{Volume of ethyl acetate extract (ml)} = \frac{60\text{mls} \times \text{Ethyl acetate extract in (mg)}}{6000\text{mg}}$$

NB: Ethyl acetate extract poorly dissolved in distilled water; hence double the volume of ethyl acetate extract was administered through the use of an oral gavage to the animals.