EFFECT OF *TETRAPLEURA TETRAPTERA* SAPONINS ON CARDIAC HISTOLOGY OF STREPTOZOTOCIN DIABETIC WISTAR RATS

BY

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ABSTRACT

Tetrapleura tetraptera (Schum. and Thonn) Taub, is a leguminous multipurpose tree (Mimosoideae) indigenous to tropical Africa. The plant has long medicinal significance as a molluscide, antimicrobial and anti-inflammatory agent. This study evaluated the effect of *Tetrapleura tetraptera* saponins on cardiac histology of Streptozotocin diabetic Wistar rats. Saponin fraction of *T. tetraptera* stem bark was orally administered to streptozotocin (STZ) diabetic rats at 10, 20 and 40mg/kg body weight (Group 4, 5 and 6). The effect of saponins on cardiac histology of the treated diabetic rats were compared with untreated control rats (Group 1), untreated diabetic control rats (Group 2) and metformin treated diabetic rats (Group 3) after 12 weeks of treatment. Treatment with graded doses of *Tetrapleura tetraptera* saponins and standard drug metformin resolved the lesions remarkably in the heart tissue with 20mg/kg body weight extract comparing favorably with metformin. There was an additional beneficial effect of vasodilation and increase in blood flow by the extract. The results from this study revealed that *Tetrapleura tetraptera* saponins ameliorated Streptozotocin induced pathology of heart tissues and may have resolved the lesions remarkably in the heart, with 20mg/kg body weight dose proving to have the best therapeutic effect.

CHAPTER ONE

1.0 INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia, which results from defects in insulin secretion, insulin action, or both (Omonkhua *et al*., 2014). The chronic hyperglycemia of diabetes is associated with long term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels (ADA, 2009). The World Health Organization (WHO) estimates that more than 180 million people worldwide have diabetes and this number is projected to double by 2030 when this disorder will affect people, irrespective of sex, age, and socioeconomic status (Wang *et al.,* 2005).

Hyperglycemia and hyperlipidemia are two important characteristics of diabetes mellitus in which diabetic patients experience various vascular complications, such as atherosclerosis, coronary heart disease, diabetic nephropathy and neuropathy (Omonkhua *et al.,* 2014). According to Framingham study, the risk factors and incidences of developing heart failure increases in diabetes mellitus regardless of hypertension, obesity, hyperlipidemia and underlying coronary heart disease (Soufy *et al*., 2012). Endomyocardial fibrosis, direct toxic effect of hyperglycaemia on cardiomyocytes, endothelial dysfunction and oxidation of low density lipoprotein could play a crucial role in both type 1 (insulin deficient) and type 2 diabetes mellitus (insulin resistant) (Soufy *et al*., 2012).

Currently, conventional drugs used for diabetes treatment are associated with drawbacks, such as rigid dosing regimens, high cost and unexpected side effects (Singh *et al.,* 2007). Therefore, screening for new anti-diabetic compounds from natural plants used in folk medicine is still

attractive, because of their efficacy, low incidence of side effects, and low cost (Omonkhua *et al.,* 2014). Although many plants have been studied for anti-diabetic and anti-hyperlipidemic effects, research still continues in this area as discoveries are always welcome for new plants with these potential and at a reduced toxicity risk. The choice to study the anti-diabetic effect of the root bark of *Tetrapleura tetraptera* was informed by the finding that local Akoko (South-West, Nigeria) traditional healers employed this part of the plant in the treatment of diabetes (Omonkhua *et al*., 2014). *Tetrapleura tetraptera* (Schum. and Thonn) Taub, is a leguminous multipurpose tree (Mimosoideae) indigenous to tropical Africa.The plant locally known in the South-Western part of Nigeria as Aridan has an age long medicinal significance as a molluscide and as an antimicrobial agent (Aderibigbe *et al.,* 2007). The aim of this study was to observe the effect of *Tetrapleura tetraptera* saponins on cardiac histology of streptozotocin diabetic Wistar rats.

The objective of this study were:

To determine the antidiabetic effects of *T. tetraptera* saponins in streptozotocin diabetic rats.

To determine the antioxidant effects of *T. tetraptera* saponins in streptozotocin diabetic rats.

To compare these effects with that obtained from a standard hypoglycaemic drug (metformin).

Justification

Previous studies has shown that *T. tetraptera* has antidiabetic, antihyperlipidemic and antioxidants effects. This study was designed to ascertain whether the results observed from previous studies are attributable to saponins.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DIABETES MELLITUS

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both (ADA, 2005). The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various 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 Beta cells of the pancreas 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 (ADA, 2005).

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 (ADA, 2005).

Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia a, and blurred vision. Impairment of growth and susceptibility to certain

infections may also accompany chronic hyperglycemia. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the nonketotic hyperosmolar syndrome (ADA, 2005).

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 (ADA, 2005).

The new classification system identifies four types of diabetes; type 1, type 2, "other specific types" and gestational diabetes (Baynes, 2015). 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 (ADA, 2005). 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 (ADA, 2005). "Other specific types," this group includes persons with genetic defects of beta-cell function (this type of diabetes was formerly called MODY or maturity-onset diabetes in youth) (Baynes, 2015). Gestational diabetes mellitus is an operational classification (rather than a pathophysiologic condition) identifying women who develop diabetes mellitus during gestation (Baynes, 2015).

2.1.1 EPIDEMIOLOGY OF DIABETES MELLITUS

The application of epidemiology to the study of diabetes mellitus has provided valuable information on several aspects of this disease such as its natural history, prevalence, incidence, morbidity and mortality in diverse populations around the world. Identification of the cause of the disease and the possible preventive measures that could be instituted to arrest or delay the onset of this disease which has reached epidemic proportions in both the developed and the developing nations (Zimmet, 1992).

The worldwide prevalence of diabetes has continued to increase dramatically. Globally, as of 2011, an estimated 366 million people had diabetes mellitus, with type 2 making up about 90% of the cases (Chen *et al*., 2012). The number of people with type 2 diabetes mellitus is increasing in every country with 80% of people with diabetes mellitus living in low- and middle-income countries. Literature search has shown that there are few data available on the prevalence of type 2 diabetes mellitus in Africa as a whole. Studies examining data trends within Africa point to evidence of a dramatic increase in prevalence in both rural and urban setting, and affecting both gender proportionally. According to the World Fact book report in 2008, in Africa the prevalence of diabetes mellitus was 3.2%, and 40,895 persons (2.0%) was in Ethiopia (Baynes, 2015).

Although type 2 diabetes mellitus is widely diagnosed in adults, its frequency has markedly increased in the pediatric age group over the past two decades. Depending on the population studied, type 2 diabetes mellitus now represents 8-45% of all new cases of diabetes reported among children and adolescents (ADA, 2000). The prevalence of type 2 diabetes mellitus in the pediatric population is higher among girls than boys, just as it is higher among women than men (Rosenbloom *et al*., 1999).

The mean age of onset of type 2 diabetes mellitus is 12-16 years; this period coincides with puberty, when a physiologic state of insulin resistance develops. In this physiologic state, type 2 diabetes mellitus develops only if inadequate beta-cell function is associated with other risk factors (e.g. obesity) (Grinstein *et al*., 2003).

Certain literatures also stated that type 1 diabetes mellitus is the most common form of diabetes in most part of the world. Wide variations exist between the incidence rates of different populations, incidence is lowest in China (0.1 per 105 per year) and highest in Finland (37 per 105 per year). In most populations, girls and boys are equally affected. In general, the incidence increases with age, the incidence peak is at puberty. After the pubertal years, the incidence rate significantly drops in young women, but remains relatively high in young adult males up to the age 29-35 years (Soltesz *et al*., 2007).

Presently as many as 50% of people with diabetes are undiagnosed. Since therapeutic intervention can reduce complications of the disease, there is a need to detect diabetes early in its course. The risk of developing Type 2 diabetes increases with age, obesity, and lack of physical activity. Its incidence is increasing rapidly, and by 2030 this number is estimated to almost around 552 million (Wild *et al*., 2004). Diabetes mellitus occurs throughout the world, but is more common (especially type 2) in the more developed countries, where the majority of patients are aged between 45 and 64 years. The greatest increase in prevalence is, however, expected to occur in Asia and Africa, where most patients will probably be found by 2030 (Wild *et al*., 2004). It is projected that the latter will equal or even exceed the former in developing nations, thus culminating in a double burden as a result of the current trend of transition from communicable to non-communicable diseases (Alberti *et al*., 1998).

2.1.2 CLASSIFICATION OF DIABETES MELLITUS

If any characteristic can define the new intentions for diabetes mellitus classification, it is the intention to consolidate etiological views concerning DM. The old and confusing terms of insulin-dependent (IDDM) or non-insulin-dependent (NIDDM) which were proposed by WHO in 1980 and 1985 have disappeared and the terms of new classification system identifies four types of diabetes mellitus: type 1, type 2, "other specific types" and gestational diabetes (Baynes, 2015).

2.1.2.1 TYPE I DIABETES MELLITUS

Type 1 diabetes mellitus (juvenile diabetes) is characterized by beta cell destruction caused by an autoimmune process, usually leading to absolute insulin deficiency (Kumar and Clark, 2002). Type 1 is usually characterized by the presence of anti–glutamic acid decarboxylase, islet cell or insulin antibodies which identify the autoimmune processes that lead to beta cell destruction. Eventually, all type1 diabetic patients will require insulin therapy to maintain normglycemia.

2.1.2.2 TYPE 2 DIABETES MELLITUS

The relative importance of defects in insulin secretion or in the peripheral action of the hormone in the occurrence of type 2 diabetes mellitus has been and will continue to be cause for discussion. Type 2 diabetes mellitus comprises 80% to 90% of all cases of diabetes mellitus (Baynes, 2015). Most individuals with Type 2 diabetes exhibit intra-abdominal (visceral) obesity, which is closely related to the presence of insulin resistance. In addition, hypertension and dyslipidemia (high triglyceride and low HDL-cholesterol levels; postprandial hyperlipidemia) often are present in these individuals. This is the most common form of diabetes mellitus and is highly associated with a family history of diabetes, older age, obesity and lack of exercise. It is more common in women, especially women with a history of gestational diabetes, and in Blacks,

Hispanics and Native Americans (Baynes, 2015).

2.1.2.3 GESTATIONAL DIABETES MELLITUS (GDM)

Gestational diabetes mellitus is an operational classification (rather than a pathophysiologic condition) identifying women who develop diabetes mellitus during gestation (Baynes, 2015). Women who develop Type 1 diabetes mellitus during pregnancy and women with undiagnosed asymptomatic Type 2 diabetes mellitus that is discovered during pregnancy are classified with Gestational Diabetes Mellitus (GDM). In most women who develop GDM; the disorder has its onset in the third trimester of pregnancy (Baynes, 2015).

2.1.3 COMPLICATION OF DIABETES MELLITUS

2.1.3.1 MICROVASCULAR COMPLICATION OF DIABETES MELLITUS

DIABETIC KIDNEY DISEASE (DKD)

Diabetic kidney disease is a common microvascular complication of diabetes mellitus, affecting approximately 25% of the diabetic population (Zelnick *et al*., 2017). Moreover, diabetes mellitus is the major cause of end stage renal disease (ESRD) in the developed world, accounting for 50% of all cases (Tuttle *et al*., 2014).

Meanwhile, there is an established relationship between albuminuria and cardiovascular disease (CVD). Specifically, microalbuminuria is considered as a risk factor for cardiovascular disease, while interventions to lower albuminuria have a positive effect on cardiovascular protection (Zeeuw *et al*., 2006). Considering the above, early diagnosis of diabetic kidney disease and appropriate intervention is of great importance (Zeeuw *et al*., 2006).

Diabetic kidney disease is defined as the presence of altered kidney function in diabetic patients, provided that other causes of chronic kidney disease are excluded. According to the American Diabetes Association’s latest guidelines, the diagnosis is based upon the findings of

decreased estimated glomerular filtration rate (eGFR <60 ml/min/1.73m2) and/or increased urinary albumin excretion (≥30 mg/g creatinine) persisting for >3 months (Tuttle *et al*., 2014).

DIABETIC RETINOPATHY

Diabetic retinopathy is the most common cause of blindness worldwide. In DR, vision loss is usually attributed to diabetic macular edema (DME) that impairs central vision, or proliferative diabetic retinopathy (PDR), that might lead to the formation of new blood vessels and fibrous tissue, resulting in fractional retinal detachment and preretinal or vitreous haemorrhage. Although diabetic retinopathy is traditionally considered as a primary vasculopathy, recent data suggest that it could be a result of diabetic retinal neurodegeneration (DRN); however, more research is required for a causal relationship to be established (Lynch and Abràmoff, 2017).

DIABETIC NEUROPATHY

Diabetic neuropathy refers to a heterogeneous group of medical conditions that affect the diabetic population with diverse clinical manifestations. It is a diagnosis of exclusion in patients with type 2 diabetes mellitus and symptoms and/or signs of peripheral nerve dysfunction (Boulton *et al*., 2004). It is the most common microvascular complication of type 2 diabetes mellitus as it affects almost 50% of patients after 10 years of disease duration, while it is estimated that 20% of diabetic patients are affected at the time of the diagnosis (Ang *et al*., 2014). Despite its high prevalence in the diabetic population the diagnosis is often missed, as almost 50% of patients are asymptomatic (Pop-Busui *et al*., 2017). If left unnoticed and untreated, the condition may result in the development of Charcot neuroarthropathy, foot ulceration and finally foot amputation with high impact on quality of life and overall life expectancy (Faselis *et al*., 2020).

SEXUAL DYSFUNCTION

Sexual dysfunction in type 2 diabetes mellitus patients is an often overlooked complication, despite the high impact of this condition on quality of life. The pathogenesis of erectile dysfunction (ED) in diabetic patients is very complex and it is a mixture of vasculopathic, neuropathic and hormonal changes that are attributed to diabetes mellitus. It is a manifestation of microangiopathy, autonomic neuropathy and macroangiopathy and as a result, erectile dysfunction could be possibly exploited as an early biomarker for diabetic complications enabling early intervention and better outcomes (Faselis *et al*., 2020).

2.1.3.2 MACROVASCULAR COMPLICATIONS OF DIABETES MELLITUS.

Patients who have type 1 diabetes tend to have coronary artery, cerebrovascular, and peripheral vascular disease more often, at an earlier age, and more extensively than the nondiabetic population. Hypertension, elevated blood lipid concentrations, and cigarette smoking are other risk factors for developing macrovascular complications (David and Leslie, 2008)

2.1.4 DIAGNOSIS OF DIABETES MELLITUS

2.1.4.1 DIAGNOSIS OF TYPE 1 DIABETES MELLITUS

A diagnosis of diabetes is based on a fasting blood glucose concentration above 7·0 mmol/L (126 mg/dL), a random blood glucose concentration above 11·1 mmol/L (200 mg/dL) with symptoms, or an abnormal result from an oral glucose tolerance test (ADA, 2018).

In the absence of symptoms, abnormal glycaemia must be present on two different occasions. A diagnosis of diabetes can also be made on the basis of a glycated haemoglobin (HbA1c) concentration above 48 mmol/mol (6·5%). However, since dysglycaemia progression can be rapid in patients with type 1 diabetes, HbA1c is less sensitive for diagnosis than fasting or stimulated blood glucose measurements (ADA, 2018).

2.1.4.2 DIAGNOSIS OF TYPE 2 DIABETES MELLITUS

Hyperglycemia is a major symptom in type 2 diabetes mellitus. Other typical symptoms of type 2 diabetes mellitus include polyuria, polydipsia, fatigue, weight loss and urine glucose. Diabetes is usually diagnosed based on plasma glucose criteria. The most widely accepted type 2 diabetes mellitus diagnostic tests are the Fasting Plasma Glucose (FPG) and the Oral Glucose Tolerance Test (OGTT) (ADA, 2010). Both FPG (diagnostic of diabetes at plasma glucose level ≥ 126 mg/dL or 7.0 mmol/L) and 2-hour OGTT (diagnostic of diabetes at plasma glucose level ≥ 200 mg/dL or 11.1 mmol/L) are commonly used diagnostic tests (ADA, 2010). The advantages of FPG are low cost and the popularity of automated laboratory machines available.

Although the OGTT has long been established as one of the diagnostic modalities for diabetes, compared with FPG, it is less practical as a plasma glucose test in clinical settings. In fact, the WHO discouraged the use of the OGTT for the diagnosis of diabetes due to its inconvenience, high cost, and poor reproducibility (ADA, 2010).

2.1.4.3 DIAGNOSIS OF GESTATIONAL DIABETES MELLITUS

Although screening procedures and diagnostic criteria vary between countries, gestational diabetes is most typically diagnosed by an oral glucose tolerance test done between 24 weeks and 28 weeks of gestation (Saravanan *et al*., 2020). Studies now recognise that the onset of gestational diabetes might occur as early as 16–20 weeks, and earlier maternal hyperglycaemia (9–10 weeks) and fetal hyperinsulinaemia (14–20 weeks) are associated with later development of gestational diabetes and a baby deemed large for gestational age(Saravanan *et al*., 2020) . Screening strategies currently recommended and offered include: universal screening using a two-step strategy (used in USA and Canada); selective risk factor screening (used in the UK); and universal screening using a one-step strategy in countries with high-risk and low-medium

risk populations (Saravanan *et al*., 2020) .

2.1.5 MANAGEMENT OF DIABETES MELLITUS

2.1.5.1 MANAGEMENT OF TYPE 1 DIABETES MELLITUS

The greatest challenge in treating type 1 diabetes mellitus is maintaining normal blood glucose level as near normal as possible whilst avoiding its large fluctuations to prevent the development of microvascular and arterial complications. Patients with type 1 diabetes mellitus are treated with insulin which can be administered in injectable, oral or possibly as inhaled forms, or with novel delivery systems based on nanotechnology (Zarogoulidis *et al*., 2011).

Management includes use of self monitoring devices for blood glucose to adjust insulin dosage and regular monitoring of risk factors to prevent diabetes associated complications (Kirti, 2012).

2.1.5.2 MANAGEMENT OF TYPE 2 DIABETES MELLITUS

A change in lifestyle, diet, weight control and a more stringent control of blood pressure and blood glucose levels are the first line defence against type 2 diabetes mellitus. In patients who do not respond to these actions and continue to show elevated blood glucose and glycated haemoglobin (HbA Ic of >6.0), oral anti-diabetic medicines are used (NCCCC, 2008).

The four major groups of anti-diabetic agents are: (a) biguanides which reduce gluconeogenesis in the liver and include metformin, (b) insulin secretagogues which stimulate the pancreas to secrete insulin and include drugs such as sulfonylureas, (c) insulin sensitizers which improve sensitivity of peripheral tissues to insulin and include thialzolidinediones and (d) insulin/insulin analogues which provide insulin exogenously in the form ofrecombinant insulin. In severe cases, these drugs are used in combination to optimise blood glucose control (Nyenwe *et al*., 2011).

2.1.5.3 MANAGEMENT OF GESTATIONAL DIABETES

Gestational diabetes mellitus can be managed through nutritional counseling consistent by the recommendation by the American Diabetes Association.

Individualization of medical nutrition therapy (MNT) depending on maternal weight and height is recommended. MNT should include the provision of adequate calories and nutrients to meet the needs of pregnancy and should be consistent with the maternal blood glucose goals that have been established. Noncaloric sweeteners may be used in moderation (ADA, 2004).

For obese women (BMI 30 kg/m2), a 30 –33% calorie restriction (to 25kcal/kg actual weight per day) has been shown to reduce hyperglycemia and plasma triglycerides with no increase in ketonuria (Franz *et al*., 2002). Restriction of carbohydrates to 35–40% of calories has been shown to decrease maternal glucose levels and improve maternal and fetal outcomes (Major *et al*., 1998).

2.2 INSULIN AND DIABETES

The mechanistic background for the diabetes is an imbalance between increased insulin requirement versus insufficient insulin availability. An important mechanism of increased insulin requirement is reduced insulin action (insulin resistance), which accompanies central body fat accumulation (Björntorp, 1993). Another mechanism is augmented glucagon secretion, which results in hyperglucagonemia andincreased glucose delivery from the liver counteracting the action of insulin (Müller *et al*., 1970). The increased insulin requirement is compensated by increased insulin secretion from the pancreatic islets, perhaps in association with increased beta cell mass. In fact, the relation between insulin sensitivity and insulin secretion is curvilinear, displaying a hyperbolic-like function (Bergman *et al*., 1981). If the islet compensation is normal, as in most subjects with insulin resistance, the hypersecretion of insulin is adequate and sufficient, which results in a well-balanced hyperinsulinemia with preserved normal glucose

tolerance. In contrast, subjects who cannot adequately increase insulin secretion in relation to the enhanced insulin demand become hyperglycemic and may develop impaired glucose tolerance (IGT) or type 2 diabetes (Bergman *et al*., 1981). Therefore,the beta cells and the pancreatic islets exert key roles for the development of type 2 diabetes.

2.3 OXIDATIVE STRESS AND DIABETES

Diabetes mellitus has been shown to be a state of increased free radical formation. As the level of ROS was high in the hyperglycemia environment, oxidative stress may be increased. Oxidative stress is believed to increase the risk of diabetes mellitus. Furthermore, since oxygen molecules are often used as a source of energy, oxidative damage cannot be avoided. Therefore, oxidative stress studies are undoubtedly useful to identify ways to prevent lifestyle-related diseases, such as diabetes (Yang *et al*., 2011).

As the hyperglycemia of diabetes becomes chronic, glucose that normally serves as metabolic substrate, fuel, and signal takes on the darker role of a toxin. Chronic hyperglycemia is the principal cause of retinopathy, kidney failure, neuropathy, and macrovascular disease in diabetes. The beta cell in T2DM is also adversely affected by chronic hyperglycemia. As hyperglycemia worsens, beta cells steadily undergoes deterioration, secrete less and less insulin, and further progress to lose their function (Yang *et al*., 2011).

Multiple biochemical pathways and mechanisms have been implicated in the deleterious effects of chronic hyperglycemia, hyperlipidemia and oxidative stress on the function of vascular, retinal, renal and islet tissues. Recent studies show that biochemical pathways through which elevated glucose concentrations can generate excessive levels of ROS (Yang *et al*., 2011). A large amount of data emphasize six key metabolic pathways as being major contributors to hyperglycemia-induced cell damage (Figure 1) (Robertson, 2004): (1) sorbitol metabolism; (2)

hexosamine metabolism; (3) dicarbonyl formation and glycation; (4) enolization and a-ketoaldehyde formation; (5) oxidative phosphorylation; and (6) protein kinase C (PKC) activation.

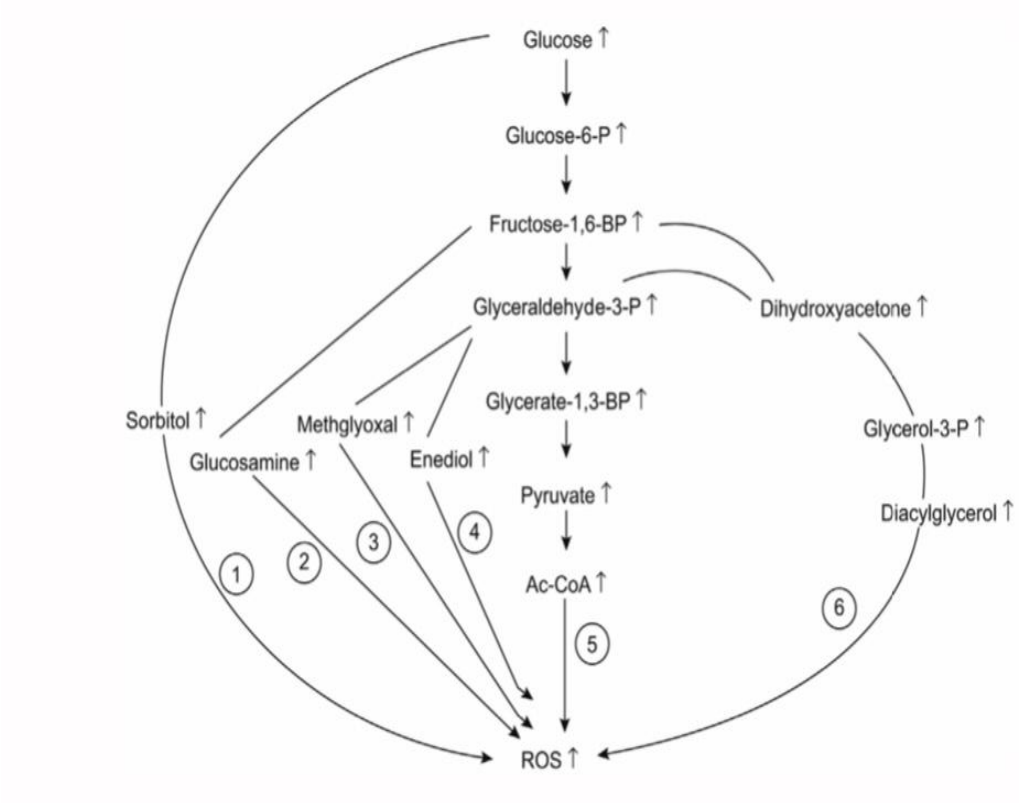

Figure 1: Hyperglycemia-induced ROS generation and consequent activation of pathological pathways (Robertson, 2004).

2.3.1 OXIDATIVE STRESS AND B-CELLS DYSFUNCTION

β-cells of pancreatic islets are more vulnerable and susceptible to the generation of ROS and oxidative stress and the levels of anti-oxidant enzyme capacity (notably, catalase, superoxide dismutase, gluthathione peroxidase and copper/zinc superoxide dismutase) in β-cells is found to

be very low as compared with other metabolic tissues notably kidney, peripheral tissues, liver and adipocytes (Rehman and Akash, 2017). Tiedge *et al*. also noted that hyperglycemia can increase cellular stress in β-cells (as measured by the increased level of heme oxygenase-1 protein), but the activities of anti-oxidant enzyme capacity was not increased which supports the notion that pancreatic islets are the most vulnerable tissues to the generation of ROS and oxidative stress (Rehman and Akash, 2017). Oxidative stress in pancreatic islets is induced by number of stimuli notably hyperglycemia, hyperlipidemia and inflammation (Garcia *et al*., 2011).

Once oxidative stress is produced, it potentiates various molecular and transcriptional pathways that are responsible to induce inflammation in pancreatic islets that ultimately leads suppress the normal functioning of IRS-1 that is responsible for the production of insulin from β-cells of pancreatic islets (Rehman and Akash, 2017).

2.4 ANTIOXIDANTS AND DIABETES

Antioxidants are structurally diverse group of small organic molecules and large enzymes that comprise complex systems of overlapping activities working synergistically to enhance cellular defense and to combat oxidative stress resulting from various reactive oxygen species (ROS) and reactive nitrogen species (RNS) (Young and Woodside, 2001). Free radicals or reactive species through oxidative stress have been evidently implicated in the incidence and progression of several health conditions such as atherosclerosis, diabetes, cancer, neurodegenerative disorders, cardiovascular disorders and other chronic conditions (Giustarini *et al*., 2009).

The interplay between the trio of free radicals antioxidants, and diseases is important in maintaining health, aging and agerelated diseases (Rahman, 2007).Input of exogenous antioxidants to human wellness has also been publicized. In fact, it has been suggested that

reduced exposure to free radicals and increased intake of antioxidant rich foods or antioxidant supplements will enhance the body's potential to minimize the risk of free radical related health problems (Lobo *et al*., 2010).

2.4.1 ENZYMATIC ANTIOXIDANTS

Antioxidant enzymes includes superoxide dismutase, catalase, glutathione peroxidases, glutathione reductases and glutathione-s-transferases have been widely investigated for the prevention and treatment of diseases resulting from oxidative damage (Hercberg *et al*., 2004).

2.4.1.1 GLUTATHIONE

Glutathione reductase and glutathione peroxidase are considered as one of the major oxidative stress biomarkers during the pathogenesis of type 2 diabetes mellitus. These enzymes are present within the cells that metabolize peroxide to water and any alteration in the levels of these enzymes make the cells more susceptible to oxidative stress (Maritim *et al*., 2003).

2.4.1.2 CATALASE (CAT)

Hydrogen peroxide (H_2O_2) in excess amount causes serious damaging effects to DNA, RNA and lipids. CAT is the main regulator of H_2O_2 and neutralizes H_2O_2 by converting it catalytically into water and oxygen. In case of deficiency of CAT, β-cells of pancreatic islets are more prone to excessive production of ROS and oxidative stress that leads to the dysfunctioning of β-cells of pancreatic islets and overt type 2 diabetes mellitus (Asmat *et al*., 2016). Recently, it has been found that chronic exposure of hyperglycemia increases the production of H_2O_2 and down-regulates the gene expression of CAT (Patel *et al*., 2013).

2.4.1.3 SUPEROXIDE DISMUTASE (SOD)

Superoxide is very harmful compound and play crucial role in the pathogenesis of type 2 diabetes mellitus (Fukai and Ushio-Fukai, 2011). Superoxide dismutase is an anti-oxidant

enzyme that is most widely present with in the body and dismutated superoxide to other compounds that are less toxic. Superoxide dismutase has the ability to breakdown the potentially harmful oxygen molecules that are present with in the cell and convert it into less toxic compound (Tiwari *et al*., 2013). It provides first line defense mechanism against ROS-induced cell injury and catalyzes the proportion of superoxide. Superoxide is a primary ROS in oxygen metabolism and Superoxide dismutase converts it into molecular oxygen and peroxide (Rehman and Akash, 2017).

2.4.2 NON ENZYMATIC ANTIOXIDANTS

Nonenzymatic antioxidants includes vitamin E, A, C, flavonoids, carotenoids, glutathione, plant polyphenols, uric acid, theaflavin, allyl sulfides, curcumin, melatonin, bilirubin, and polyamines. Some of these antioxidants are water-soluble and predominantly found in the cytosol or cytoplasmic matrix, while others are liposoluble and are present in cell membranes (Nimse and Pal, 2015). Dietary antioxidants have been hypothesized to have a protective effect against the development of diabetes by inhibiting peroxidation chain reactions (Montonen *et al*., 2004).

2.5 DIABETOGENES

Diabetogenes are diabetogenic agent that produces a persistent elevation in blood glucose concentration to within the values accepted by the Report of the International Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (Gavin, *et al*.,1997), i.e.,when obtained on repeat measurements on different days, a casual plasma-glucose concentration of 11.1 mmol/L in the presence of symptoms (polyuria, polydipsia, unexplained weight loss), a fasting plasma-glucose concentration of 7.0 mmol/L, or a 2 hour post-prandial plasma-glucose concentration of 11.0 mmol/ L.

2.5.1 ALLOXAN

In 1838, Wöhler and Liebig synthesised a pyrimidine derivative, which they later called alloxan (Lenzen, 2008). In 1943, alloxan became of interest in diabetes research when Dunn and McLetchie reported that it could induce diabetes in animals as a result of the specific necrosis of the pancreatic beta cells (Lenzen, 2008). The resulting insulinopenia causes a state of experimental diabetes mellitus called 'alloxan diabetes' (Lenzen, 2008). The reduction product of alloxan, dialuric acid, has also been shown to be diabetogenic in animals, and to cause ultrastructural changes identical to those observed in response to alloxan (Lenzen, 2008).

2.5.1.1 ALLOXAN: MECHANISM OF ACTION

Alloxan has two distinct pathological effects: it selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell, and it causes a state of insulin-dependent diabetes through its ability to induce ROS formation, resulting in the selective necrosis of beta cells. These two effects can be assigned to the specific chemical properties of alloxan, the common denominator being selective cellular uptake and accumulation of alloxan by the beta cell (Lenzen, 2008).

2.5.1.1.1 BETA CELL SELECTIVITY OF ALLOXAN

Both alloxan and glucose are hydrophilic and do not penetrate the lipid bilayer of the plasma membrane. The alloxan molecule is structurally so similar to glucose that the GLUT2 glucose transporter in the beta cell plasma membrane accepts this glucomimetic and transports it into the cytosol (Lenzen, 2008). Alloxan does not inhibit the function of the transporter, and can therefore selectively enter beta cells in an unrestricted manner (Lenzen, 2008). It is therefore not toxic to insulin-producing cells that do not express this transporter (Elsner *et al*., 2002). The half-life of alloxan is short; in aqueous solution it spontaneously decomposes into non-diabetogenic

alloxanic acid within minutes (Lenzen, 2008). Because of this, it must be taken up and accumulated quickly in the beta cell, and is therefore ineffective when blood flow to the pancreas is interrupted for the first few minutes after alloxan injection (Lenzen, 2008). N-substituted alloxan derivatives with a long carbon side chain, such as butylalloxan, differ chemically from alloxan in that they are lipophilic (Lenzen, 2008). Butylalloxan acts in a similar manner to alloxan and preferentially damages beta cells (Lenzen, 2008). But since derivatives such as butylalloxan are lipophilic they can also penetrate plasma membranes that do not express the GLUT2 transporter (Elsner *et al*., 2002). Nephrotoxicity is a dominating feature of the toxicity of lipophilic derivatives after systemic administration (Lenzen, 2008). This nephrotoxicity is so severe that it causes fatal renal failure in the animals before diabetes can develop (Lenzen, 2008). The susceptibility of the kidney to the toxic action of these lipophilic alloxan derivatives is the result of their preferential accumulation in the tubular cells of the kidney, which, like the beta cells, express the GLUT2 glucose transporter (Lenzen, 2008).

2.5.2 STREPTOZOTOCIN

Streptozotocin is an antimicrobial agent and has also been used as a chemotherapeutic alkylating agent In 1963, Rakieten et al. reported that streptozotocin is diabetogenic (Lenzen, 2008). Again, this insulinopenia syndrome, called ‘streptozotocin diabetes’, is caused by the specific necrosis of the pancreatic beta cells and streptozotocin has been the agent of choice for the induction of diabetes mellitus in animals ever since (Lenzen, 2008).

2.5.2.1 STREPTOZOTOCIN: MECHANISM OF ACTION

Streptozotocin inhibits insulin secretion and causes a state of insulin-dependent diabetes mellitus. Both effects can be attributed to its specific chemical properties, namely its alkylating potency. As with alloxan, its beta cell specificity is mainly the result of selective cellular uptake

and accumulation (Lenzen, 2008).

2.5.2.1.1 BETA CELL SELECTIVITY OF STREPTOZOTOCIN

Streptozotocin is a nitrosourea analogue in which the N-methyl-N-nitrosourea (MNU) moiety is linked to the carbon-2 of a hexose. The toxic action of streptozotocin and chemically related alkylating compounds requires their uptake into the cells. Nitrosoureas are usually lipophilic and tissue uptake through the plasma membrane is rapid; however, as a result of the hexose substitution, streptozotocin is less lipophilic. Streptozotocin is selectively accumulated in pancreatic beta cells via the low-affinity GLUT2 glucose transporter in the plasma membrane (Lenzen, 2008). Thus, insulin-producing cells that do not express this glucose transporter are resistant to streptozotocin (Lenzen, 2008). This observation also explains the greater toxicity of streptozotocin compared with N-methyl-N-nitrosourea in cells that express GLUT2, even though both substances alkylate DNA to a similar extent (Elsner *et al*., 2000). The importance of the GLUT2 glucose transporter in this process is also shown by the observation that streptozotocin damages other organs expressing this transporter, particularly kidney and liver (Lenzen, 2008).

2.5.2.1.2 BETA CELL TOXICITY OF STREPTOZOTOCIN

It is generally assumed that the toxicity of streptozotocin is dependent upon the DNA alkylating activity of its methylnitrosourea moiety, especially at the O_6 position of guanine (Lenzen, 2007). The transfer of the methyl group from streptozotocin to the DNA molecule causes damage, which along a defined chain of events, results in the fragmentation of the DNA (Lenzen, 2008). Protein glycosylation may be an additional damaging factor (Konrad and Kudlow, 2002). In the attempt to repair DNA, poly(ADP-ribose) polymerase (PARP) is overstimulated. This diminishes cellular NAD+, and subsequently ATP, stores (Lenzen, 2008). The depletion of the cellular energy stores ultimately results in beta cell necrosis. Although

streptozotocin also methylates proteins, DNA methylation is ultimately responsible for beta cell death, but it is likely that protein methylation contributes to the functional defects of the beta cells after exposure to streptozotocin.

Inhibitors of poly ADP-ribosylation suppress the process of DNA methylation. Thus, injection of nicotinamide and other PARP inhibitors in parallel with, or prior to the administration of streptozotocin is well known to protect beta cells against the toxic action of streptozotocin and to prevent the development of a diabetic state (Lenzen, 2008). Also, mice deficient in PARP are resistant to beta cell death mediated by streptozotocin, in spite of DNA fragmentation. The absence of PARP prevents the depletion of the cofactor NAD+ and the subsequent loss of ATP and thus cell death (Lenzen, 2008).

The role of alkylation in beta cell damage has also been examined by the use of ethylating agents, which are less toxic than their methylating counterparts, on account of O_6- ethylguanine being less toxic than O_6 -methylguanine (Lenzen, 2008). The fact that N-ethyl-N-nitrosourea and ethyl methanesulphonate are significantly less toxic to insulin-producing cells than MNU and methyl methanesulphonate has been taken as support for the notion that in insulin producing cells, as in other cell types, the mechanism of toxic action is due to alkylation, with methylation of DNA bases being more toxic than ethylation (Lenzen, 2008). An alternative hypothesis proposes that part of the diabetogenic effect of streptozotocin may relate not to its alkylating ability but to its potential to act as an intracellular nitric oxide (NO) donor (Lenzen, 2008). Both streptozotocin and MNU contain a nitroso group and can liberate NO. In fact, streptozotocin has been shown to increase the activity of guanylyl cyclase and the formation of cGMP, which are characteristic effects of NO. However, the alkylating agent methyl methanesulphonate is the most toxic compound, though unlike MNU, it is not a NO donor, indicating that NO is not an

indispensable prerequisite for the toxic action of the family of alkylating agents that streptozotocin belongs to. Finally, some minor generation of ROS, including superoxide and hydroxyl radicals originating from hydrogen peroxide dismutation during hypoxanthine metabolism, may accompany the effect of streptozotocin and accelerate the process of beta cell destruction but ROS do not play a crucial role (Lenzen, 2007).

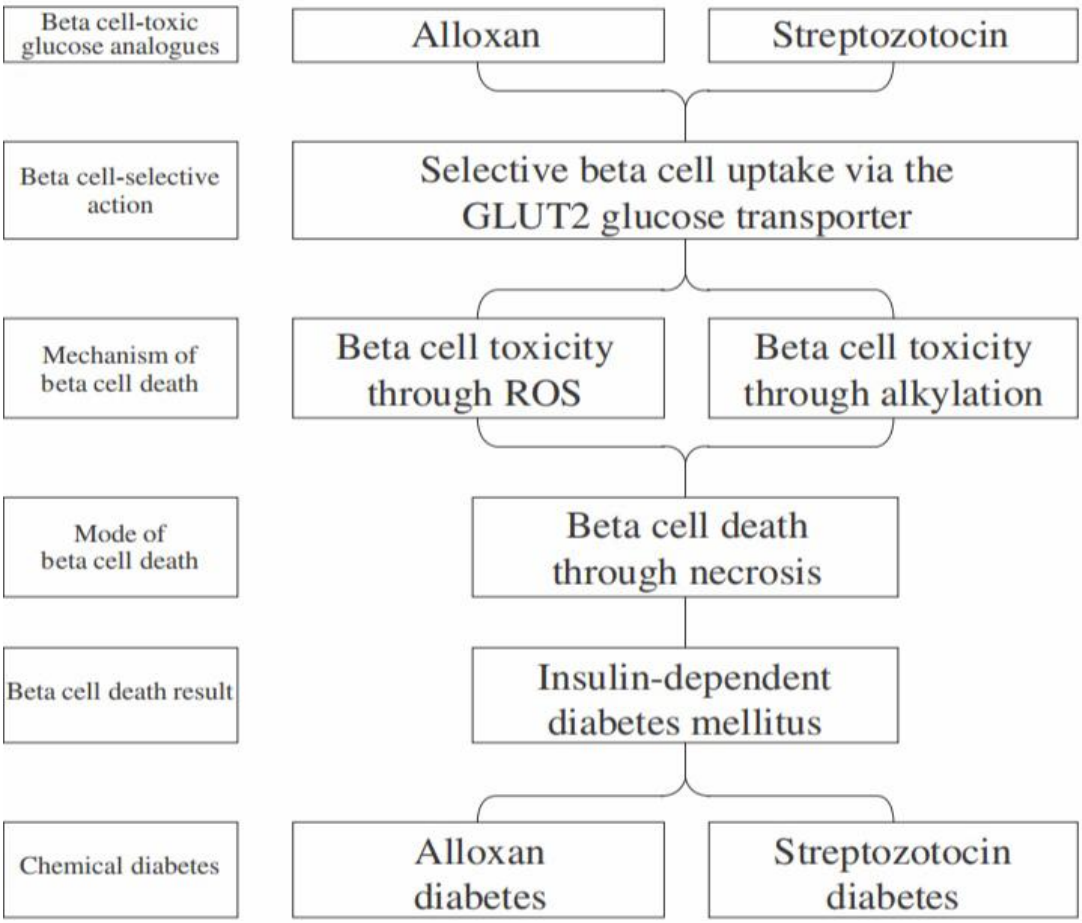

Figure 2: Schematic representation of the toxic effects of the glucose analogues alloxan and streptozotocin in beta cells, which produce chemical diabetes (Lenzen, 2008).

2.5.3 COMPARING ALLOXAN WITH STREPTOZOTOCIN AS DIABETOGENIC AGENTS

Streptozotocin has notable advantages over alloxan as chemical agents or induction of experimental diabetes, thus, is often preferred to the latter (alloxan). For instance, streptozotocin has longer half-life (15 min against 1.5 min of alloxan) (Macdonald *et al*., 2017). This makes it more stable in solution before and after injection into animals. Streptozotocin induced hyperglycemia is relatively more stable and for a longer duration (as much as three months compared to alloxan-induced hyperglycemia that can only be sustained for less than a month). Moreover, the mechanism of streptozotocin diabetogenicity is less associated with cellular toxicity, hence, lesser animal mortality. Alloxan on the contrary, induces diabetes by a mechanism characterized by incidences of ketosis, reactive oxygen species toxicity (ROS), and high mortality rate which is particularly a major setback in experimental diabetes studies (Szkudelski, 2001). One reason for this is that streptozotocin is more selective to islet beta cells than alloxan which causes severe damage to other cell types which express GLUT2 (systemic toxicity) (Macdonald *et al*., 2017).

More so, streptozotocin-induced diabetes is associated with well characterized diabetic complications unlike alloxan-induced diabetes (Lenzen, 2008). In addition, compared to alloxan, streptozotocin diabetogenicity is not severely interfered with by blood glucose level. Overall, streptozotocin diabetogenicity is more effective and with lesser variation with animal species (Macdonald *et al*., 2017).

2.6. *TETRAPLEURA TETRAPTERA*

Tetrapleura tetraptera, a popular medicinal plant belonging to the family Fabaceae (formerly Leguminosae: Mimosoideae), is commonly known as “Aridan” or “Aidan” among the Yoruba ethnic group of South Western Nigeria, “Abogolo”among the Igala people of north central Nigeria, and “Dawo”among the Hausa people of Northern Nigeria. It is generally found

in the lowland forest of many tropical African countries, and known to have fruit that consists of fleshy pulp and small, brownish-black seeds, a characteristic fragrant and pungent aromatic odor (Aladesanmi, 2007).

It is used as a popular seasoning spice in Southern and Eastern Nigeria, and its fruit is used for the management of convulsions, leprosy, inflammation, rheumatism, flatulence, jaundice and fevers, as well as the management and control of adult onset type 2 diabetes mellitus (Odesanmi *et al*., 2011).

Compared with other commonly used spices, it is a rich source of phytochemicals which contribute to its documented biological and pharmacological activities, including cardiovascular, antiinflammatory, hypoglycaemic, hypotensive, neuromuscular, anti-convulsant, molluscicidal, trypanocidal, hirudinicidal, anti-ulcerative, ectoxicity, anti-microbial, emulsifying property, birth control, food value and the control of intestinal parasites (Akin-Idowu *et al*., 2011). The nutrients and anti-nutrients content of *Tetrapleura tetraptera* fruits have also been reported (Akin-Idowu *et al*., 2011). The phytochemical composition in the fruits of *Tetrapleura tetraptera* includes polyphenols (tannins, flavonoids), saponins, phytate, triterpenoid, coumarinic (scopoletin) and phenolic (caffeic acid, cinnamic acids) compounds, a triterpene glycoside (aridanin) which have been found as the active ingredients (Akin-Idowu *et al*., 2011).

Tetrapleura tetraptera for example have been reported to contain triterpenoidal saponins and flavonoids (Ojewole and Adewunmi, 2004). One of the most obvious features of the aqueous root bark of *T. tetraptera* is the very high degree of frothing, which is a classic indicator of the presence of saponins. Polyphenols, including saponins and flavonoids, apart from their antioxidant properties, are reported to exert hypoglycaemic effects. Plant flavonoids have been reported to produce hypoglycaemia by reducing glucose absorption, acting as insulin

secretagogues or insulin mimetics and/or stimulating glucose uptake in peripheral tissues (Brahmachari, 2011).

In addition, triterpenoidal saponins have been reported to increase glucose uptake by tissue and cause pancreatic β cell regeneration (Koneri *et al*., 2014). Though the exact mechanism by which T. *tetraptera* exerts its antidiabetic effect remains unknown; the presence of these phytochemicals in *T. tetraptera* may be responsible for its anti-diabetic effect.

2.7 SAPONIN AND DIABETES

Saponins are amphipathic glycosides secondary metabolites which synthesized by many different plant species, have high molecular weight, consisting of a sugar moiety united to a triterpenoid or steroid sapogenins. Saponin has received numerous attention due to their various biological activities that including hepatoprotective, antitumor, antimicrobial, and anti-inflammatory activities. Marine organisms such as starfish, sponges and sea cucumbers are now considered a rich source of saponin (Moghimipour *et al*., 2015).

Saponins have been known to possess the anti-diabetic property and are promising compounds with potential to be developed into new drugs for anti-diabetes (Kim *et al*., 2006).

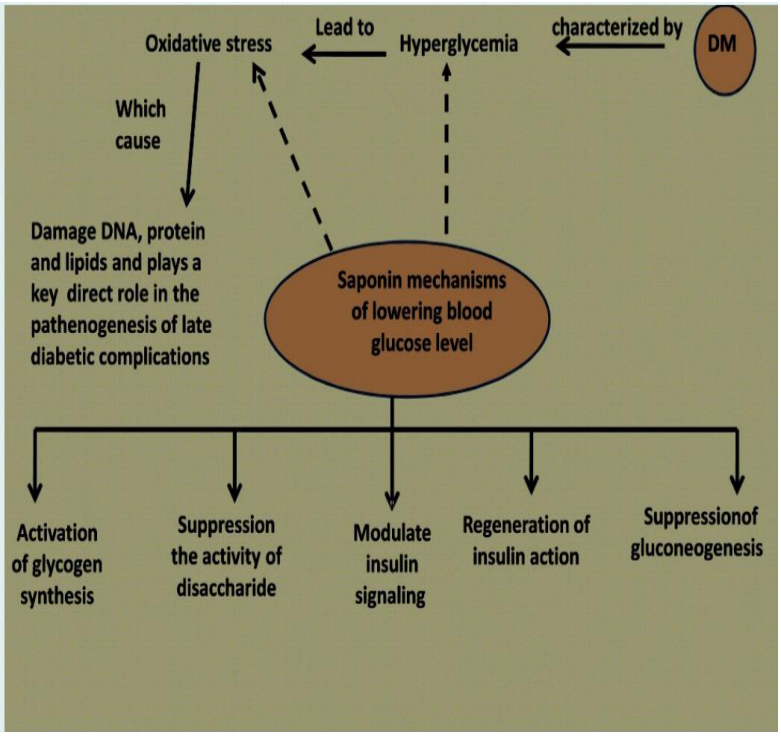

Figure 3: Mechanism of action of saponin in diabetes (El Barky *et al*., 2017).

2.7.1 CHEMICAL STRUCTURE OF SAPONIN

Saponins chemically consist of two parts aglycone and glycone, the aglycone part is also known by sapogenin which classified to either triterpenoid (C-30), neutral or alkaloid steroids (C-27) (Karimi *et al*., 2011). The aglycone part covalently linked to one or more glycone (sugar), which may be glucose, galactose, glucuronic acid, xylose or rhamnose, the oligosaccharide is attached at the C3 position but in some saponins,additional sugars are attached at C26 or C28 positions (Francis *et al*., 2002).

2.8 OVERVIEW OF THE HISTOLOGY OF THE HEART

The heart is a four-chambered organ responsible for pumping throughout the body. It receives deoxygenated blood from the body, sends it to the lung, receives oxygenated blood from the lungs, and then distributes the oxygenated blood throughout the body. At the histological

level, the cellular features of the heart play a vital role in the normal function and adaptations of the heart.

The cells that constitute the heart are unique. It can initiate and propagate electricity throughout each cardiac cell. This physiology allows the heart to contract synchronously, permitting for optimal function of circulating blood to the lungs and the rest of the distal organs.

2.8.1 STRUCTURE

The fibrous skeleton, cardiac muscle, and impulse conduction system constitute the basic framework of the heart. The base of the heart contains a highly dense structure known as the fibrous or cardiac skeleton. Functions of the fibrous skeleton include providing as a strong framework for cardiomyocytes, anchoring the valvular leaflets, and acting as electrical insulation separating the conduction in the atria and ventricles (Saremi *et al*., 2017).

The wall of the heart separates into the following layers: epicardium, myocardium, and endocardium. These three layers of the heart are embryologically equivalent to the three layers of blood vessels: tunica adventitia, tunica media, and tunica intima, respectively. A double-layer, fluid-filled sac known as the pericardium, surrounds the heart. The two layers of the pericardium are called the outer fibrous/parietal pericardium and the inner serous/visceral pericardium. The epicardium constitutes the visceral pericardium, underlying fibro-elastic connective tissue, and adipose tissue (Rodriguez and Tan, 2017). Coronary arteries and veins, lymphatic vessels and nerves run below the epicardium. The endocardium is composed of the endothelium and the subendothelial connective tissue layer. The subendocardium is found between the endocardium and myocardium and contains the impulse-conducting system.

The impulse conducting system has specialized cardiac cells for the conduction of electrical impulses throughout the heart. Electrical impulses initiate at the sinoatrial (SA) node, situated at

the junction of the superior vena cava and right atrium. These impulses travel throughout the atria until it reaches the atrioventricular (AV) node; located between the interatrial and interventricular septum. As the fibers travel inferiorly, it penetrates the central fibrous body of the cardiac skeleton to form the bundle of His. These fibers are the Purkinje fibers after they divide within the interventricular septum and branch into the ventricles.

Valves are an important component of the heart. Not only do they act as an exit gate, but they also prevent backflow into the chamber. The aortic valve, separating the aorta from the left ventricle, and the pulmonic valve, separating the pulmonary artery from the right ventricle, are known as semilunar valves. The two atrioventricular (AV) valves are the tricuspid and mitral valves. The tricuspid valve marks the separation between the right atrium and right ventricle while the mitral valve separates the left atrium from the left ventricle. A unique aspect of the AV valves is their attachments to sthe ventricles with the assistance of chordae tendinae inserting onto the papillary muscle of the ventricles.

2.8.2 FUNCTION

The heart's main function is to pump blood throughout the body. Cardiac function can be best represented by cardiac output, the amount of blood pumped out of the heart per minute. Many factors determine the cardiac output. The product of stroke volume and heart rate equals cardiac output. Hence, cardiac output is directly alterable through variations in these two factors. Stroke volume is the blood volume ejected after ventricular contraction, calculated by taking the difference between end-diastolic volume and end-systolic volume. Contractility, afterload, and preload can change stroke volume.

Preload is the amount of stress placed on cardiomyocytes by the end-diastolic volume before systole. The end-diastolic volume is the best way to measure preload. On the other hand,

afterload is the total tension exerted onto the ventricle that must overcome during systole. The law of LaPlace is the foundation for the definition of afterload. Therefore, changes in pressure, radius, or wall thickness directly affect afterload (Norton, 2001).

2.9 JUSTIFICATION

Previous study demonstrated the in vivo antidiabetic, antihyperlipidaemic and antioxidant effects of aqueous extracts of *T. tetraptera* root bark. The presence of saponins is easily observable in *T. tetraptera* root bark. Several reports have shown that saponins from different sources have antidiabetic, antihyperlipidaemic and antioxidant properties; this study is thus designed to ascertain whether the results observed from our previous study are attributable to saponins. This will help narrow down the phytochemicals that exert the observed potential therapeutic effects of *T. tetraptera* root bark, which can ultimately lead to the elucidation of the exact antidiabetic substance in this plant.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.1 PLANT COLLECTION

Tetrapleura tetraptera (schum. And thonn) Taub was obtained from Akungba-Akoko, Ondo State, South-Western Nigeria and identified in the Department of Plant Science and Biotechnology, University of Benin, Nigeria. Hebarium specimen was deposited at the Hebarium of the University of Benin, Nigeria. The plant materials were washed thoroughly under running water, shade dried abd then pulverized.

3.1.2 CHEMICALS AND REAGENT PREPARATION

All chemicals were of an analytical grade and are suppkied from Sigma Chemicals Co. USA. Distilled water was used in all biochemical assays.

1. Streptozotocin.
2. Formalin.
3. Paraffin.
4. Hematoxylin.
5. Eosin.

3.1.3 EQUIPMENT

1. Microscope.
2. Freeze dryer.
3. Electronic digital weighing balance.
4. Electronic sensitive weighing balance.
5. Glucometer.

3.2 METHODS

The scheme below was employed for the extraction of *T. tetraptera* total saponins (TTS) fractions.

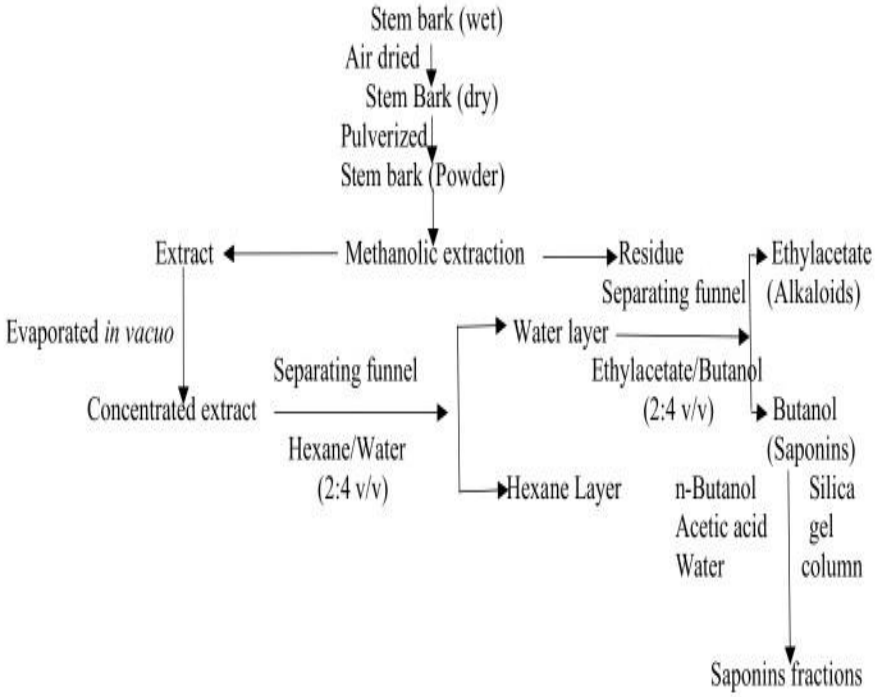

Figure 4: Method used to fractionate saponins from *T. tetraptera* stem bark, adapted from Hostettmann *et al*., (1991)

The method used to fractionate saponins from T. tetraptera stem bark was adapted from Hostettmann *et al.* (1991). The plant sample (material) was harvested, collected and washed thoroughly under running water, shade dried, pulverized (crushed to powder) and then sieved. 1134g of the powdered plant sample was soaked in 4800ml of methanol (methanol extract) for 72 hours (3 days) in a glass container and covered with foil paper. At the end of the third day, it was filtered through two layers of cheesecloth and later with a filter paper to completely remove residues. The filtrate was concentrated by a rotary evaporator and then evaporated to dryness by means of a freeze dryer. The dried extract was homogenized by means of a laboratory mortar and pestle, and then weighed.

The homogenized extract (methanol extract) was extracted with n-hexane and water in a ratio of 1:2 (v/v) in a separating funnel and left for 48 hours (2 days) on a retort stand. At the end

of the second day, the mixture was decanted into two fractions; water layer and hexane layer. The water layer (aqueous extract) was concentrated by a rotary evaporator and then evaporated to dryness by means of a freeze dryer. The dried extract (aqueous extract) was homogenized by means of a laboratory mortar and pestle, and then weighed.

The homogenized extract (aqueous extract) was extracted with ethylacetate and butanol in a ratio of 1:2 (v/v) in a separating funnel, and left for 48 hours (2 days) on a retort stand. At the end of the second day, the mixture was decanted into two fractions; butanol layer and ethylacetate layer. The butanol layer (butanol extract), which contains saponin was concentrated by a rotary evaporator and then evaporated to dryness by means of a freeze dryer. The dried extract (butanol extract), which is saponin was homogenized by means of a laboratory mortar and pestle, weighed and stored in an air-tight container and kept in the freezer until use.

3.2.3 PREPARATION AND ADMINISTRATION OF PLANT EXTRACT

The *T. tetraptera* total saponins (TTS) fractions was concentrated under pressure and then freeze dried; the TTS was reconstituted appropriately in distilled water. Different doses of TTS and metformin, at 100 mg/kg body weight was administered orally (by *gavage*) daily for 12 weeks.

3.2.4 INDUCTION OF DIABETES

Streptozotocin was dissolved in acidified (pH 4.5) normal saline and administered to the rats, by intra-peritoneal injection, at a dose of 65mg/kg body weight after a 12-hour fast. Diabetes was confirmed after seven (7) days of STZ administration by measuring fasting blood sugar (FBS). After stable diabetes was established (FBS > 180 mg/dl), TTS and metformin administration to rats was commenced and lasted for 12 weeks.

3.3 ANIMALS AND EXPERIMENTAL PROTOCOL

A total of forty-two (42) adult male rats of the Wistar strain, with average weight of 120 g were purchased from the Department of Animal Science, University of Ibadan, Nigeria. They were be kept in a well aerated room (Department of Anatomy Animal House, University of Benin), with 12h light and 12h dark cycles. They were fed twice a day (standard pelleted feed) and given water *ad libitum*. The animals were acclimatize for two weeks before the commencement of the study. Treatment of the animals conformed to the guidelines for the Care and Use of Laboratory Animals (NAS, 2011). An ethical clearance on animal handling and care with the code, EC/FP/020/19 was obtained for this study.

3.3.1 PERIODIC DETERMINATION OF THE WEIGHT OF ANIMALS

The weight of the animals were determined using an electronic weighing balance every week to measure the change in body weight over the period of administration.

3.3.2 BLOOD COLLECTION

Blood for monitoring fasting blood sugar was drawn from the tail vein of each rat once in two weeks and assessed with a glucometer. At the end of 12 weeks, the rats were sacrificed by decapitation; blood was collected through the trunk. The thoracic/abdominal regions were opened to expose the heart and other organs. Blood for glucose assays (2 mls) was collected in fluoride bottles while that for other biochemical analyses (2 mls) was collected in plain bottles. The blood samples were allowed to clot on ice and centrifuged at 1,000 X g for 5 minutes; the serum was separated for analysis.

3.3.3 TISSUE HOMOGENIZATION

The liver, heart, kidneys, and pancreas were removed and blotted with cotton wool. Portions for tissue homogenization were washed in ice cold normal saline, and homogenized in ice cold normal saline 4:1 w/v. The homogenate was centrifuged at 1,000 X g for 5 minutes; the

separated supernatant was stored in a freezer until analysis.

3.3.4 TISSUE HISTOLOGY

Heart was fixed in 10% buffered formalin and dehydrated by graded series of alcohol, embedded in paraffin, sectioned at 5 μm in thickness, and will be cut using a microtome and mounted on glass slides. Heart sections were stained with hematoxylin and eosin (H and E). The sections were examined under a microscope with digital camera attached.

In vivo studies, using the heart of streptozotocin diabetic rat were done to ascertain the antidiabetic and antioxidant effects of *T. tetraptera* saponins (TTS). This phase of the study consisted the following:

The induction of experimental diabetes in rat models – normal and streptozotocin diabetic rats were divided into the following groups of nine (9) rats each:

a. Normal control (untreated normal rats)

b. Diabetic control (untreated diabetic rats)

c. Positive control (diabetic rats treated with metformin)

d. Diabetic rats treated with 10 mg/kg body weight of TTS

e. Diabetic rats treated with 20 mg/kg body weight of TTS

f. Diabetic rats treated with 40 mg/kg body weight of TTS

The extracts were administered orally for twelve (12) weeks (by *gavage*), after which the rats were sacrificed. Histological evaluation of the heart was conducted.

CHAPTER FOUR

4.0 RESULTS

4.1 HISTOPATHOLOGICAL ASSESSMENT OF THE HEART TISSUE

The heart histology was examined after inducing diabetes in Wistar rats using streptozotocin and treating with graded doses (10, 20, and 40 mg/kg b.w) of *Tetraptera*

tetraptera saponins (TTS) for 90 days.

Across the diabetic groups, ~~administration of~~ streptozotocin-induced alterations in the blood vessels of the heart which manifested ~~as~~ **in** complications ~~including;~~ **such as** erosions and ulceration was observed. In the heart, it induced perivascular inflammation (Vasculities).

Treatment with the standard drug metformin and graded doses of TTS resolved the lesions remarkably in the heart tissue, with 20mg/kg body weight extract comparing favourably with metformin. The histology of the heart tissues in groups 4, 5 and 6 (groups treated with graded doses of 10, 20 and 40 mg/kg b.w saponin extract revealed vasodilation with the concomitant increase in blood flow. ~~This suggests that the extract could be beneficial in treating high blood pressure, a complication of diabetes~~.

Diabetes has been well documented as a cause of vasculopathy (atherosclerosis), damaging the walls of blood vessels. Group 1 (i.e the untreated streptozotocin-induced group) exhibited a weak and negative reaction in the blood vessels which is indicative of vasculopathy (atherosclerosis).

The heart tissue of a normal control Wistar rat showed myocardiac fibre bundles and ovoid nuclei containing normal chromatic arrangement and interstitial spaces which are histological indications of a normoglycemic state. This is shown in figure 4.1 below:

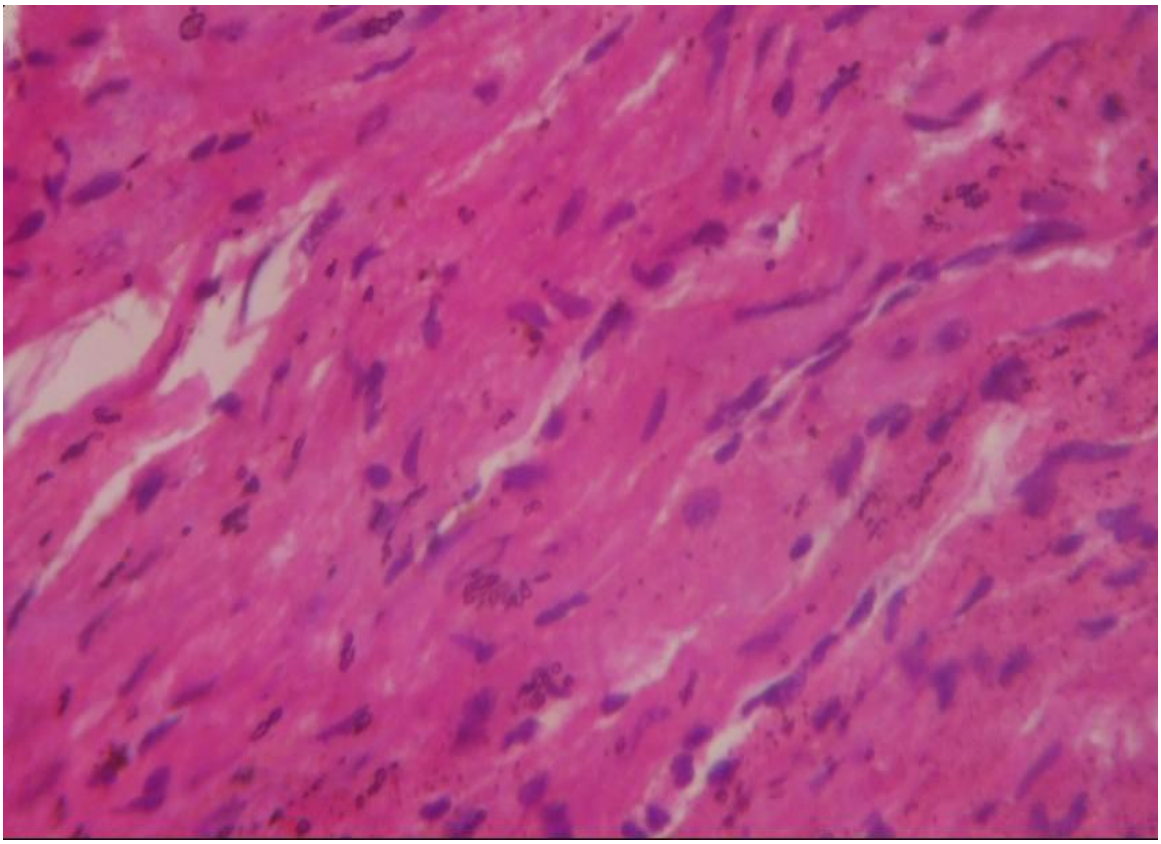

FIG 4.1: Group 1 (Normal Control) Heart tissues. A, myocardiac fibre bundles, B, ovoid nuclei containing normal chromatic arrangement, C, interstitial space (H&E x 400)

The heart tissue of a Wistar rat administered with streptozotocin (but was untreated) showed coronary artery with intimal ulceration and perivascular infiltrates of inflammatory cells which are histological indications of a diabetic state. This is shown in figure 4.2 below:

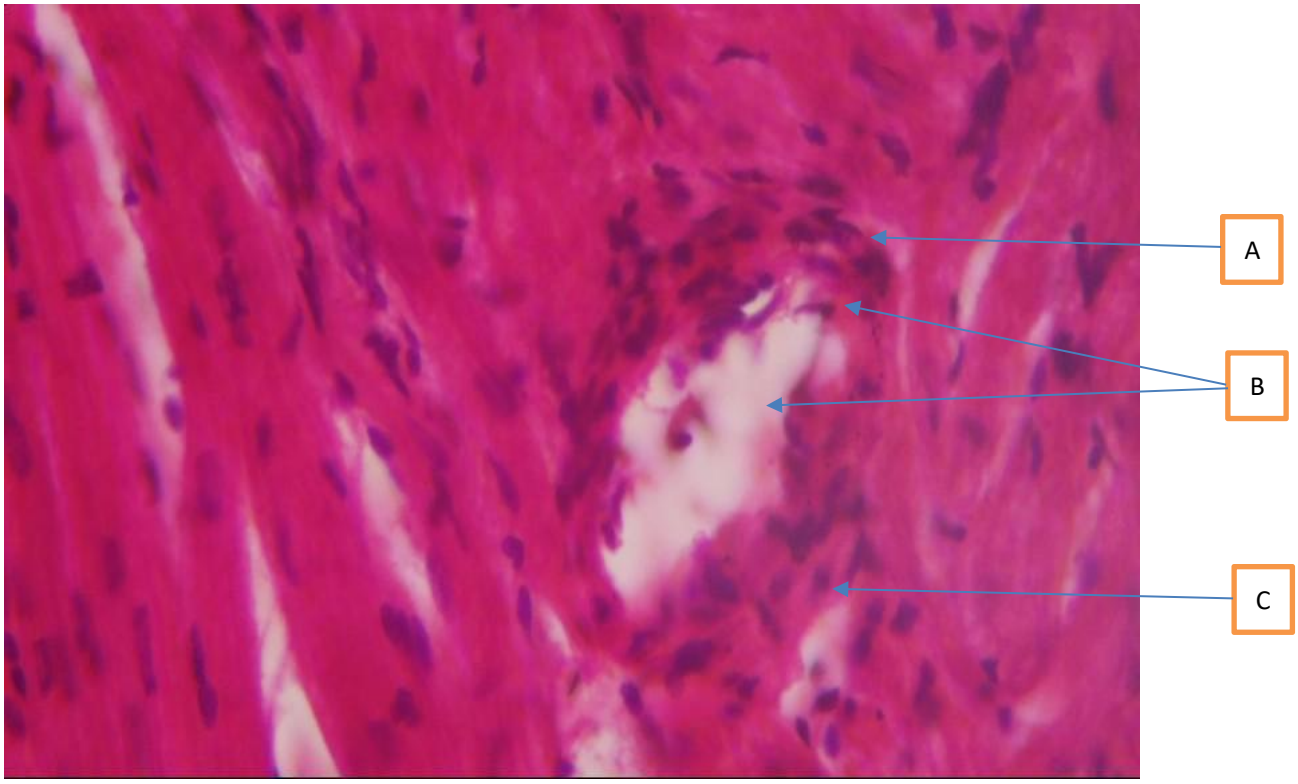

FIG 4.2: Group 2 (Diabetic Control). Heart tissue of Streptozotocin-induced diabetic rat. A, coronary artery with B, intimal ulceration and C, perivascular infiltrates of inflammatory cells (H&E x 400)

The heart tissue of a Wistar rat administered with streptozotocin but treated with metformin showed normal myocardiac fibre bundles and dilated coronary artery which histologically indicates an amelioration of a diabetic condition. This is shown in figure 4.3 below:

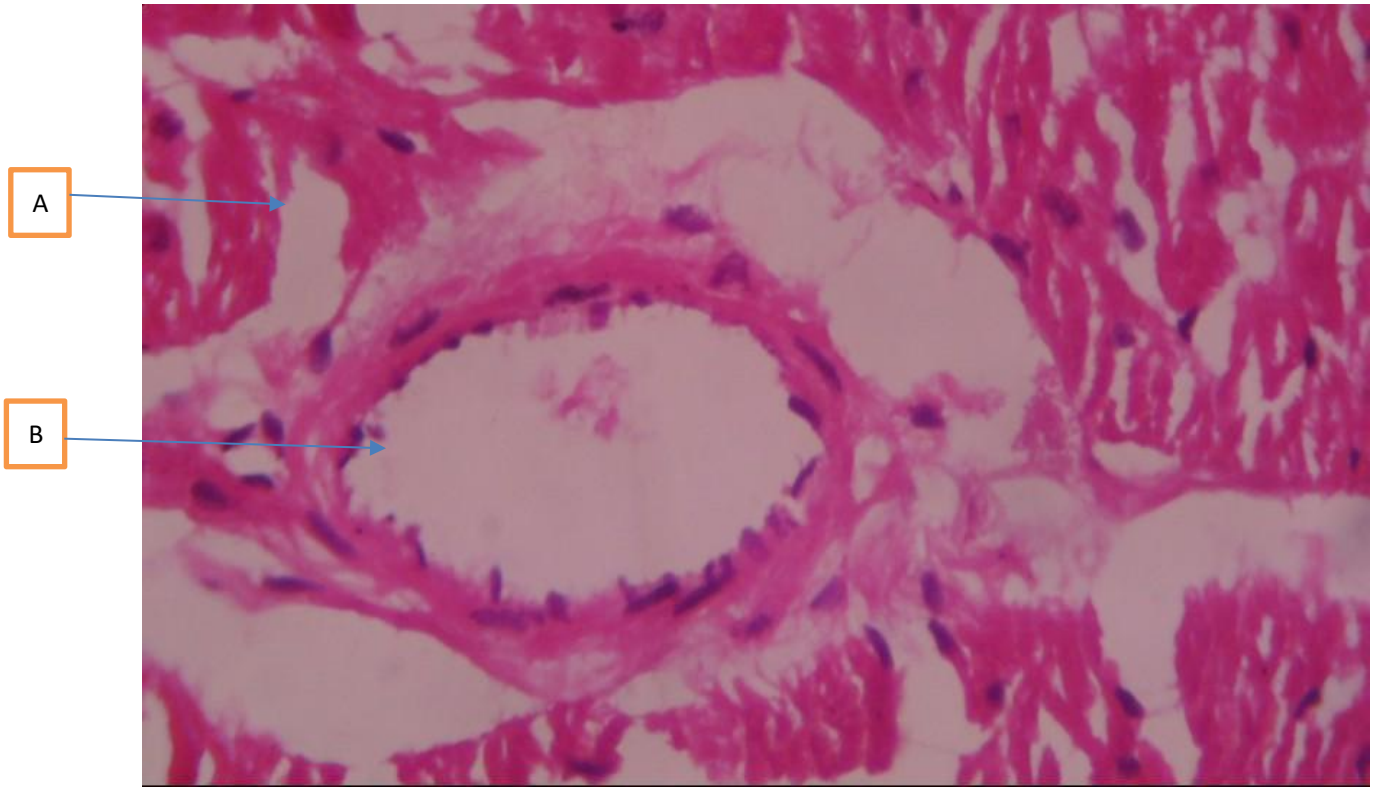

FIG 4.3: Group 3 (Metformin treated streptozotocin-diabetic rats). A, normal myocardiac fibre bundles and B, dilated coronary artery (H&E x 400)

The heart tissue of a Wistar rat administered with streptozotocin but treated with 10mg/kg b.w of *T. tetraptera* saponin showed normal myocardiac fibre bundles, vascular dilatation and active congestion which histologically indicates an amelioration of a diabetic condition. This is shown

in figure 4.4 below:

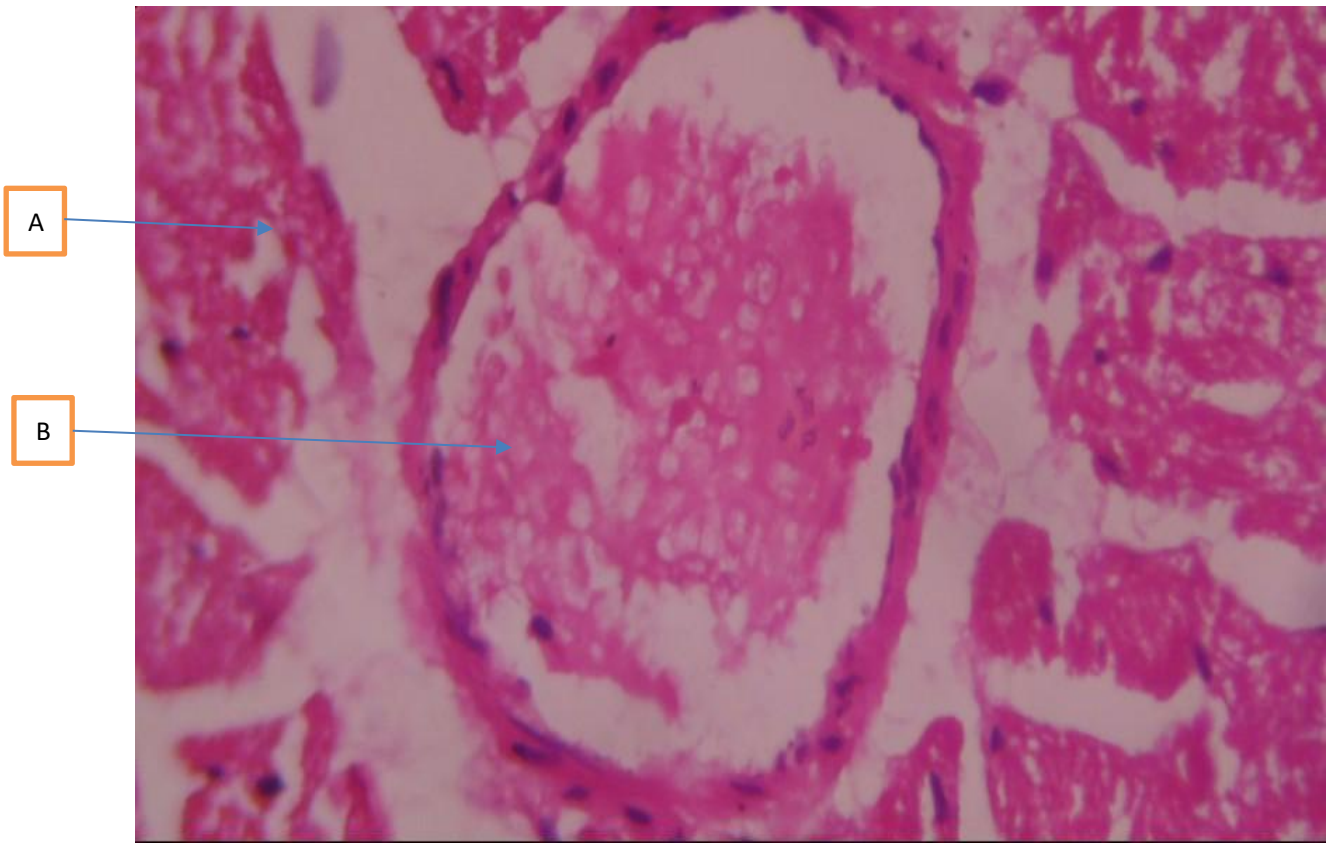

FIG 4.4: Group 4 (STZ diabetic rats treated with 10 mg/kg body weight of TTS).: A, normal myocardiac fibre bundles and B, vascular dilatation and active congestion (H&E x 400)

The heart tissue of a Wistar rat administered with streptozotocin but treated with 20mg/kg b.w of *T. tetraptera* saponin showed normal myocardiac fibre bundles, vascular dilatation and active congestion which histologically indicates an amelioration of a diabetic condition. This is shown

in figure 4.5 below:

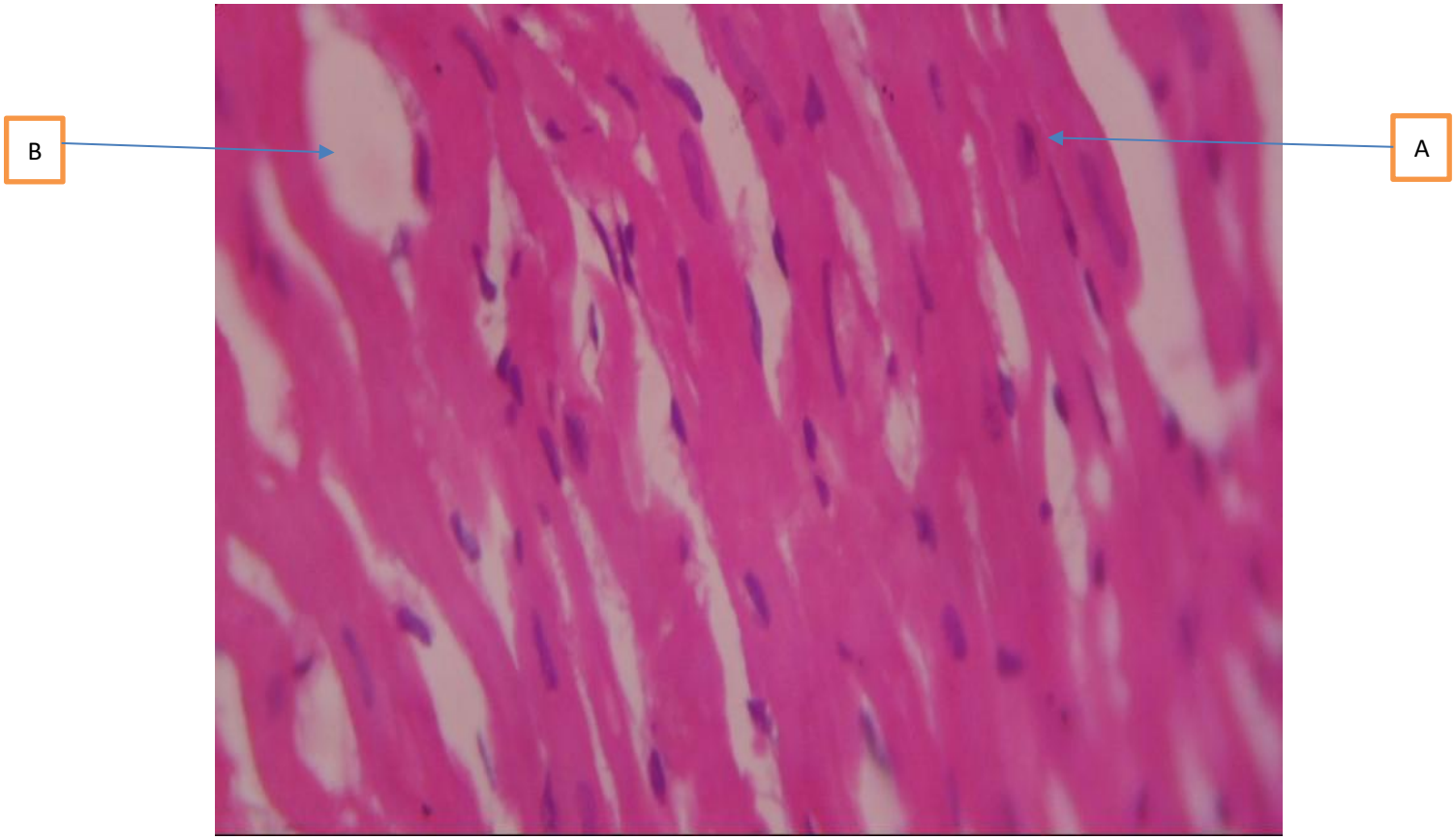

FIG 4.5: Group 5 (STZ diabetic rats treated with 20 mg/kg body weight of TTS): A, normal myocardiac fibre bundles and B, vascular dilatation (H&E x 400)

The heart tissue of a Wistar rat administered with streptozotocin but treated with 40mg/kg b.w of *T. tetraptera* saponin showed normal myocardiac fibre bundles and vascular stenosis which histologically indicates an amelioration of a diabetic condition. This is shown in figure 4.6 below:

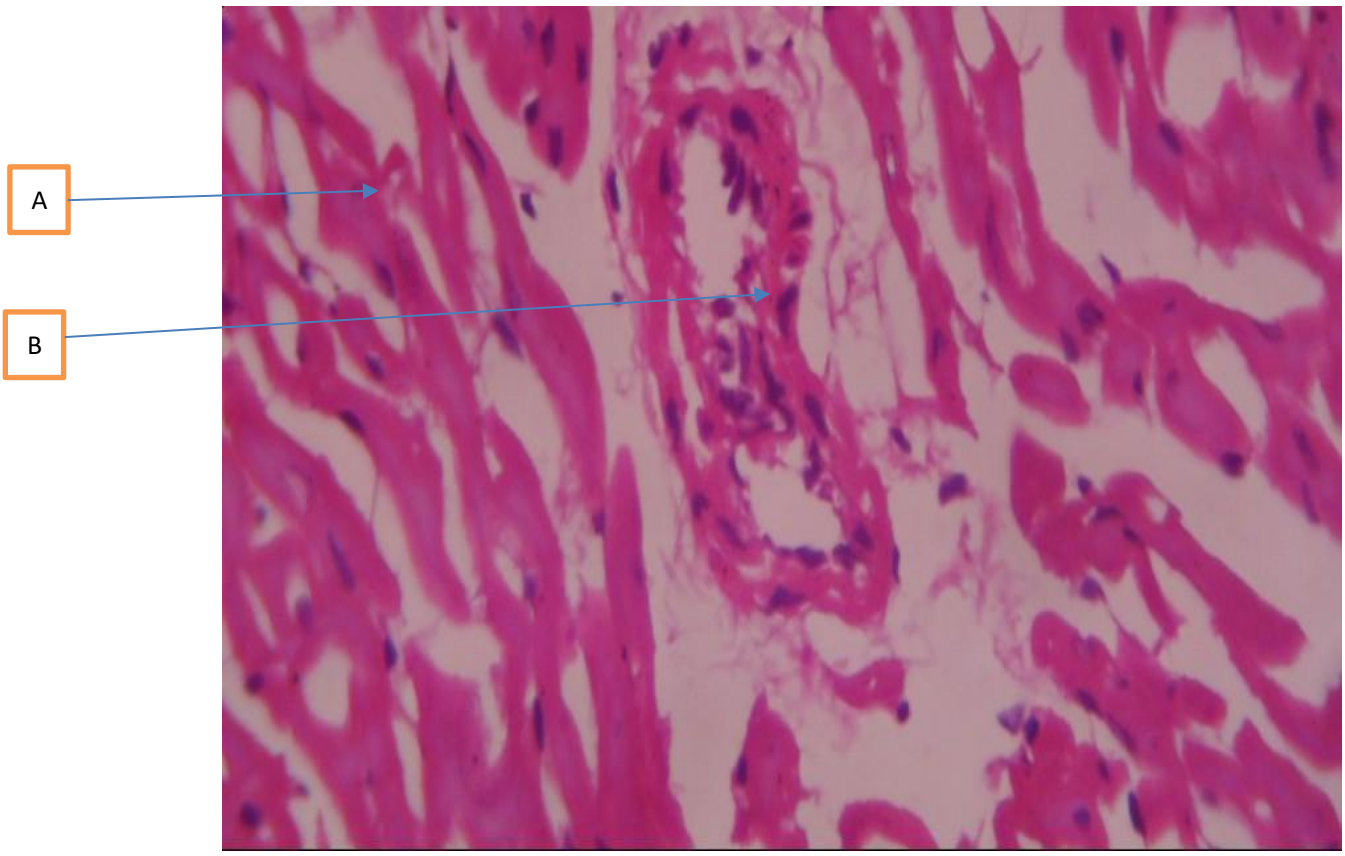

FIG 4.6 Group 6 (STZ diabetic rats treated with 40 mg/kg body weight of TTS): A, normal myocardiac fibre bundles and B, vascular stenosis (H&E x 400)

CHAPTER 5

5.0 DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Streptozotocin induces diabetes in experimental animals by destruction of the pancreatic β-cells which results in the elevation of the blood glucose level in the diabetic rats (Beppu *et al*., 2006). In this study, the fasting blood sugar (FBS) concentration of all groups injected with STZ increased significantly compared to normal control. The FBS concentration of the untreated diabetic group remained high for the duration of the study indicating that STZ produced stable experimental diabetes (Howarth *et al*., 2005).

Saponins are well known bioactive phytocompounds, and have been investigated for a multitude of activities, including antimicrobial, cytotoxic, anti-inflammatory, hypolipidemic, immune stimulatory and antidiabetic (Francis *et al*., 2002). In this study, we observed the effect of *Tetrapleura tetraptera* saponins on cardiac histology of streptozotocin diabetic Wistar rats. The fruits of *T. tetraptera* have been reported to contain triterpenoidal saponins and flavonoids (Ojewole and Adewunmi, 2004). One of the most obvious features of the aqueous root bark of *T. tetraptera* is the very high degree of frothing, which is a classic indicator of the presence of saponins. Polyphenols, including saponins and flavonoids, apart from their hypoglycemic properties, are reported to exert antioxidants effects (Omonkhua *et al.*, 2014). One of the most obvious effects of saponins is its activity on lipid hydroperoxide and oxidative stress (Rodrigues *et al*., 2005).

Various theories have been proposed that cardiovascular damage is the result of oxidative stress process (Droge, 2002) and the most important risk indicators for cardiovascular alterations are increased serum total cholesterol, TG, LDL and decreased serum levels of HDL (Abuja and Albertini, 2001). The coexistence of hypertension and diabetes increase the risk of developing macrovascular complications (myocardial infarction, stroke) and also microvascular complications (nephropathy and retinopathy) (Matheus *et al*., 2013).

Diabetes has been documented to cause vasculopathy (atherosclerosis), damaging the walls of blood vessels (Basta *et al*., 2004). In this study, there was a weak and negative reaction in the blood vessels in the untreated streptozotocin diabetic group which manifested as complications including; erosions and ulceration. In the heart, it induced perivascular inflammation (Vasculities). This study also shows significant positive effect of 20mg/kg BW TTS extract in reducing the negative effect of STZ. The effect of 20 mg/Kg body weight TTS compares favorably with metformin in resolving the lesions in the heart. There was an additional beneficial effect of vasodilation and increase in blood flow by TTS which is critical in ameliorating high blood pressure which complicates diabetes. The proposed mechanisms that can link accelerated atherosclerosis and increased cardiovascular risk in subjects with diabetes are still poorly understood (Matheus *et al*., 2013); but it appears that TTS may be beneficial in addressing this lesion.

The findings of this study revealed that *T. tetraptera* saponins ameliorated STZ induced pathology of heart tissues and may have resolved the lesions remarkably in the heart. The presence of saponins, which have been demonstrated to possess antioxidants effect, may be responsible for these findings (Francis *et al*., 2002). This antioxidant effect could be useful in managing diabetes induced cardiovascular complications.

5.2 CONCLUSION

The ameliorated effect of streptozotocin-induced pathology of heart tissues may be attributed to the presence of saponin fraction of *Tetrapleura tetraptera* stem bark and this effect may play

important roles in the management of diabetes-induced cardiovascular complications, with 20mg/kg body weight dose proving to have the best therapeutic effect.

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