EVALUATION OF THE ANTIOXIDANT PROPERTIES OF METHANOL EXTRACT OF THE LEAVES OF *Justicia carnea*

BY

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CERTIFICATION

This is to certify that this research work was carried out by **EGBON OSAMUDIAMEN** with matriculation number **LSC1806278** of the Department of Biochemistry, Faculty of life science, University of Benin, as part of the requirements for the award of Bachelor of Science (B.Sc) degree in Biochemistry.

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DEDICATION

This work is dedicated to my parents Mr. and Mrs Egbon for their unwavering support for my academic success.

ACKNOWLEDGEMENT

I am grateful to Almighty God for his grace, love and mercy, and to my parents Mr. and Mrs. Egbon for their unwavering support for my academic success.

Special appreciation goes to my supervisor, Dr. Sam Ojeaburu for his guidance, scholarly contribution to the success of my project and fatherly disposition towards me throughout the period of this work.

Sincere thanks to my siblings and friends for being there for me.

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ABSTRACT

Justicia carnea is a medicinal plant with varied pharmacological effects. The present study evaluated the antioxidant properties of methanol extract of the leaves of *Justicia carnea* in Wistar rats. Mature Wistar albino rats (n = 36) were assigned to six groups of 6 rats each: group 1 (normal control), group 2 (diabetic control), group 3 (diabetic rats treated with standard anti-diabetic drug, metformin), group 4 (diabetic rats + 100mg/kg bwt of methanol extract of *Justicia carnea*), group 5 (diabetic rats + 200mg/kg bwt extract) and group 6 (diabetic rats + 500mg/kg extract). The plant leaves were extracted with absolute methanol. Diabetes mellitus was induced in the rats via intraperitoneal injection of 120 mg/kg body weight STZ. Fasting blood glucose (FBG), body weight, acute toxicity and oxidative stress markers were measured. Acute toxicity study showed that at the highest dose of 5000 mg/kg bwt, methanol extract of *J. carnea* did not produce any mortality in the rats. Graded doses of methanol extract of *J. carnea* leaves significantly reduced the blood glucose concentration of streptozotocin-induced diabetic rats ($p < 0.05$). The greatest weight increase was observed in the control group when compared to the other groups ($p < 0.05$). The plant extract significantly increased the activities of catalase, superoxide dismutase (SOD), and glutathione peroxidase (GPx), but it reduced the concentration of malondialdehyde (MDA) significantly when compared with the diabetic control group ($p < 0.05$). This study has provided evidence to support the claim that methanol extract of *J. carnea* is effective in ameliorating oxidative stress induced by STZ in diabetic Wistar rats.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The human body comprises of cells that require energy. This energy is gotten from food and broken down into simpler units. The primary food source is glucose, a simple sugar gotten from the breakdown of carbohydrates. It is important that the levels of glucose in the body are regulated, that is regulation of glucose production and utilization. Hormones such as insulin and glucagon play a role in glucose metabolism. These hormones are produced by the pancreas located at the back of the abdomen behind the stomach. At high glucose levels, insulin acts as a doorway to drive glucose into cells stimulating glucose utilization while glucagon does the opposite, it makes glucose available for cells from glycogen, stimulating glucose production, and this happens at low glucose levels in the blood. Glycogen is the storage form of glucose when there is more than enough sugar in the blood. Ingestion of carbohydrates brings about a quick rise in insulin concentration and a decrease in glucagon concentration. (Ripsin *et al.*, 2009). When there's not enough insulin produced or the presence of abnormal insulin secretion, glucose levels become extremely high resulting in hyperglycemia, an indication for diabetes. This causes the kidneys to filter and absorb the excess glucose into the urine taking along fluids from the body, leading to dehydration (Ripsin *et al.,* 2003). Another indication of diabetes mellitus is a condition called insulin resistance, caused by elevated levels of free fatty acids and inflammatory cytokines in the plasma. These inflammatory factors include tumor necrosis factor (TNF), C-reactive protein, interleukin- 6(IL-6), adiponectin etc. these inflammatory factors bring about a

connection between obesity and inflammatory diseases including type-2 diabetes mellitus (Ripsin *et al.,* 2003).

Diabetogenic agents such as alloxan and streptozotocin (STZ) are used to induce diabetes in test compounds by necrosis of the beta cells. Numerous medicinal plants have been recommended for the treatment of diabetes mellitus, and the anti-diabetic properties of some plants have also been reported by researchers.

Plants have been used in traditional medicines since time immemorial. It has been estimated that 70-80% of world population relies on herbal medicine for their primary healthcare needs (Adenuga *et al.,* 2020). Medicinal plants such as *Justicia carnea* have been used against various diseases for thousands of years, and 80% of the worldwide population still depends on herbal medicines (Parsaeimehr *et al.,* 2017; Akinyemi *et al.,* 2018). *Justicia* is the largest genus of Acanthaceae family, having about six hundred species (Akinyemi *et al.,* 2018). *Justicia* is a creeping annual or perennial herb growing up to 1.5 or 2 metres tall. This is one of the most appreciated of the sweet green vegetables of Africa. It is cultivated in home gardens in West and Central Africa, especially in Nigeria, Guinea, Ghana, Togo, Benin, Sierra Leone, Cameroon and DR Congo (Osioma and Hamilton- Amachree, 2017; Tropical Plants Database, 2020). Many species of *Justicia* have been used traditionally in treating respiratory tract infection, inflammation, gastrointestinal disorders (Onyeabo *et al.,* 2017; Ukpabi-Ugo *et al.,* 2019a; Ani *et al.*, 2020; Onochie *et al*., 2020). fever, diabetes (Ukpabi-Ugo *et al.,* 2017), liver diseases, diarrhea, arthritis, rheumatism, anaemia (Wood *et al.,* 2020),, cancer, HIV and diabetes. In DR Congo, species of *Justicia* have also been reported to be use in treating various illnesses such as cancers, arthritis, eye disease, diabetes, gastrointestinal disease, HIV, fever, vaginal discharge,

epilepsy, dyspepsia, mental disorder, headache, inflammation. They are also used as analgesics, sedatives, hallucinogens, depressors and somniferous agent (Khan *et al.,* 2017).

Also many metabolites have been reported present in the genus *Justicia*, which include flavonoids, alkaloids, diterpenoids, vitamins, triterpenoids, iridoids, steroids, coumarin, lignans and triterpenoidal glycosides (Aziz *et al.,* 2017). Lignans have been reported to be major components of most extracts of species of *Justicia* and have shown some pharmacological actions such as antiinflammatory, anti-tumoral, anti-platelet aggregation, hepatoprotective, male contraceptive, hallucinogenic, superoxide anion radical scavenging, nephroprotective, haematinic, antimicrobial, anti sickling, immunomodulatory, antihypertensive and antiviral activities (Arogbodo, 2020). Though there are many reported ethnomedicinal uses of the *J. carnea*, there are not many scientific researches to support those claims.

1.1.1 Aim and Objectives of Study

The aim of this study was to evaluate the antioxidant properties of methanol extract of the leaves of *Justicia carnea* in Wistar rats.

The specific objectives were to:

i. Extract the leaves of *J. carnea* with absolute methanol

ii. Induce diabetes mellitus in Wistar rats using STZ

iii. Estimate blood glucose in rat blood

iv. Estimate the activity and concentration of antioxidants in rats

1.2 Literature Review

1.2.1 Diabetes Mellitus

Diabetes mellitus is a metabolic disorder characterized by the presence of hyperglycemia linked to defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes mellitus is related to extended damage and failure of various organs like the eyes, kidneys, heart and blood vessels. It is necessary to understand the biological process that occurs during and after a meal, in order to make sense of diabetes. When food is introduced into the body, it breaks down into simpler units and gets absorbed into the bloodstream. When carbohydrates are broken down, it forms glucose that's absorbed into the bloodstream and carried to tissues like liver, muscle where it is needed for energy. The presence of this sugar signals the pancreas to secrete the hormone, insulin. Insulin allows this glucose to enter the cells, providing energy for cell functioning (Roussel, 1998). Diabetes mellitus has been known by physicians for about 3500 years in ancient Egypt, it was defined as a condition in which a person urinates excessively and loses weight (Polonsky, 2012).

Complications of the affected organs include:

Eyes: cataracts that is gradual damaging of the eye that could lead to blindness

Cardiovascular system: stroke, hardening of the arteries

Kidneys: kidney failure

Nerves: neuropathy

In 1936, the difference between type 1 and type 2 diabetes mellitus was made clear. Type 1 diabetes also known as insulin-dependent diabetes mellitus is caused by the destruction of the

pancreatic beta cells. Type 2 diabetes mellitus formerly known as adult-onset diabetes is caused by a defect in insulin action (Albright,1997).

1.2.1.2 Types Of Diabetes Mellitus

1.2.1.2.1 Type 1 diabetes Mellitus (T1DM)

Data on global trends in T1DM prevalence and incidence are not available, but data from many high-income countries indicate an annual increase of between 3 % and 4 % in the incidence of T1DM in childhood. Males and females are equally affected. Despite T1DM occurring frequently in childhood, onset can occur in adults and 84 % of people living with T1DM are adults. Type 1 diabetes mellitus decreases life expectancy by around 13 years in high income countries. The prognosis is far worse in countries with limited access to insulin. Distinguishing Type 1 diabetes mellitus and Type 2 diabetes mellitus(T2DM) in adults can be challenging, and misclassifying T1DM as T2DM and vice versa may impact estimates of prevalence and incidence. A recent study applied a T1DM genetic risk score to individuals of European descent taking part in the UK's Biobank research project and concluded that 42 % of T1DM occurred after the age of 30 years and accounted for 4 % of all cases of diabetes diagnosed between the ages of 31 and 60 years. The clinical characteristics of these individuals included a lower body mass index, use of insulin within 12 months of diagnosis, and increased risk of diabetic ketoacidosis (Davis and Granner, 1996)

The rate of β-cell destruction is rapid in some individuals and slow in others. The rapidly progressive form of T1DM is commonly observed in children but may also occur in adults. Some patients, particularly children and adolescents, may present with ketoacidosis as the first manifestation of the disease. Others may have modest 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 β-cell function sufficient to prevent ketoacidosis for many years. At the time of classical clinical presentation with T1DM, there is little, or no insulin secretion as manifested by low or undetectable levels of C-peptide in blood or urine. The presence of obesity in people with T1DM parallels the increase of obesity in the general population. Between 70 % and 90 % of people with T1DM at diagnosis have evidence of an immune-mediated process with β-cell autoantibodies against glutamic acid decarboxylase (GAD65), islet antigen-2 (IA-2), ZnT8 transporter or insulin, and associations with genes controlling immune responses, in populations of European descent, most of the genetic associations are with HLA DQ8 and DQ2. The specific pathogenesis in those without immune features is unclear, although some may have monogenic forms of diabetes. These two groups of T1DM have previously been referred to as type 1A (autoimmune) and type 1B (non-immune) diabetes but this terminology is not frequently used nor is it clinically helpful. Consequently, this report refers only to T1DM without the subtypes used in the WHO 1999 classification. Fulminant type 1 diabetes is a form of acute onset T1DM in adults mainly reported in East Asia. It accounts for approximately 20 % of acute-onset T1DM in Japan and 7 % in Korea. It is also common in China but rare in people of European descent. The major clinical characteristics of fulminant type 1 diabetes include abrupt onset; very short duration (usually less than 1 week) of hyperglycemic symptoms; virtually no C-peptide secretion at the time of diagnosis; ketoacidosis at the time of diagnosis; mostly negative for islet-related autoantibodies; increased serum pancreatic enzyme levels; frequent flu-like and gastrointestinal symptoms just before the disease onset (Davis and Granner, 1996). Cellular infiltration of macrophages and T cells into islets suggests an accelerated immune response to virus infected islet cells and rapid destruction of β-

cells. Measuring islet autoantibodies remains important to research as it can help shed light on the etiology and pathogenesis of T1DM. While measuring islet autoantibodies has limited value in clinical practice, in classical T1DM it may have a role when there is uncertainty as to whether a person has T1DM or T2DM. However, the decision to use insulin should not rely on the presence of such markers, but rather on the clinical need (Rother, 2007).

1.2.1.4 Type 2 Diabetes Mellitus

Type 2 diabetes mellitus accounts for between 90 % and 95 % of diabetes, with highest proportions in low- and middle-income countries. It is a common and serious global health problem that has evolved in association with rapid cultural, economic, and social changes, ageing populations, increasing and unplanned urbanization, dietary changes such as increased consumption of highly processed foods and sugar sweetened beverages, obesity, reduced physical activity, unhealthy lifestyle and behavioral patterns, fetal malnutrition, and increasing fetal exposure to hyperglycemia during pregnancy. Type 2 diabetes mellitus is most common in adults, but an increasing number of children and adolescents are also affected β-cell dysfunction is required to develop T2DM. Many with T2DM have relative insulin deficiency and early in the disease absolute insulin levels increase with resistance to the action of insulin. Most people with T2DM are overweight or obese, which either causes or aggravates insulin resistance. Many of those who are not obese by basal metabolic intake criteria have a higher proportion of body fat distributed predominantly in the abdominal region, indicating visceral adiposity compared to people without diabetes. However, in some populations, such as Asians, β-cell dysfunction appears to be a more notable prominent than in populations of European descent (Dorner, 1977). This is also observed in thinner people from low and middle-income countries such as India, and among people of Indian descent living in high income countries. For most people with T2DM,

insulin treatment is not required for survival but may be required to lower blood glucose to avert chronic complications. Type 2 diabetes mellitus often remains undiagnosed for many years because the hyperglycemia is not severe enough to provoke noticeable symptoms of diabetes. Nevertheless, these people are at increased risk of developing macrovascular and microvascular complications. Complications are a particular problem in young-onset T2DM – increasingly recognized as a severe phenotype of diabetes and associated with greater mortality rates, more complications, and unfavorable cardiovascular disease risk factors when compared to T1DM of similar duration. In addition, the response to oral blood glucose medications is often poor among young people with diabetes. Many factors increase the risk of developing T2DM including age, obesity, unhealthy lifestyles, and prior gestational diabetes (GDM). The frequency of T2DM also varies between different racial and ethnic subgroups, especially in young and middle-aged people. There are populations that have a higher occurrence of type 2 diabetes, for example Native Americans, Pacific Islanders, and populations in the Middle East and South Asia. It is also often associated with strong familial, likely genetic or epigenetic predisposition (Krishnasamy and Abell, 2018).

1.2.1.5 Impaired Fasting glucose and Impaired Glucose Tolerance (IFG and IGT)

Impaired fasting glucose and Impaired glucose tolerance represent intermediate states of abnormal glucose regulation that exist between normal glucose homeostasis and diabetes. IFG is now defined by an elevated fasting plasma glucose (FPG) concentration (>100 and <126 mg/dl). Impaired fasting glucose is defined by an elevated two-hour plasma glucose concentration (>140 and <200 mg/dl) after a 75g glucose load on the oral glucose tolerance test (OGTT) in the presence of fasting plasma glucose concentration (Genuth *et al*., 2003). The combined characteristics of IFG and IGT have been studied by identifying populations that fulfill both

criteria (FPG=100–125 mg/dl and 2hour value = 140–199 mg/dl). Conversely, normal glucose tolerance (NGT) is defined as fasting plasma glucose (FPG) <100 mg/dl and 2hour plasma glucose <140 mg/dl, Impaired fasting glucose was defined in 1997 by the American Diabetes Association as a means of classifying individuals who had fasting glucose levels between normal and diabetes. It was meant to be analogous to IGT as an intermediate metabolic state between normal and diabetes, but based on the fasting plasma glucose. (Cowie *et al*., 2006).

Impaired fasting glucose and Impaired glucose tolerance also differ significantly in their age and sex distribution; the prevalence of both metabolic disorders increases with advancing age. Impaired glucose tolerance is more frequent in women than in men. The natural history of both IFG and IGT is variable, with 25 % progressing to diabetes, 50 % remaining in their abnormal glycemic state, and 25 % reverting to no glucose tolerance test over an observational period of 3–5 years. Individuals who are older, overweight, and have other diabetes risk factors are more likely to progress. Moreover, low insulin secretion and severe insulin resistance identify individuals more likely to progress to diabetes. With longer observation, most individuals with IFG or IGT appear to develop diabetes. Both IFG and IGT have a heterogeneous pathogenesis, and this may contribute to different rates of progression to diabetes. Also, the poor precision and accuracy of glucose measurements and the poor reproducibility of the glucose tolerance test itself contribute to the difficulty of defining the natural history of IFG/ IGT in any one individual. Individuals with both IFG and IGT have approximately double the rate of developing diabetes compared with individuals with just one of them. However, with recent changes in the cut point defining IFG, the risk of develop range (110– 125 mg/dl) was changed in 2003 to 100– 125 mg/dl so that the population risk of developing diabetes with IFG would be like that with IGT. The change in the cut point increased the overall prevalence of IFG approximately three to four-

fold. It is clear, however, that IGT and IFG do not define the same individuals. The prevalence of IFG and IGT varies widely, with recent data from the U.S. indicating the prevalence of IFG to be 26 % and somewhat older data showing a 15% prevalence of IGT. Both are expected to increase in the foreseeable future. Numerous longitudinal studies indicate that both IFG and IGT are associated with a modest increase in the hazard ratio (1.1–1.4) for cardiovascular disorder with IGT being a slightly stronger risk predictor. The majority of this risk appears to be conferred by progression to diabetes, when the risk of cardiovascular disorder increases two to four-fold. Many cardiovascular risk factors (for example, low high-density lipoprotein (HDL) cholesterol, hypertension, and elevated triglycerides) are prevalent in IFG and IGT, but it is unclear whether they occur more frequently in one state than the other. However, after adjustment for known cardiovascular risk factors, both IFG and IGT remain as independent, albeit weak, risk factors for cardiovascular disorder in some studies but not in others. Even so, it is unclear whether the cardiovascular disorder risk associated with IFG or IGT can be attributed to the development of diabetes during follow up or whether these states per se convey such risk (David *et al*., 2007).

The epidemiologic differences between IFG and IGT suggest that different pathophysiologic mechanisms contribute to these disturbances in glucose homeostasis. During a standard 75g OGTT, people with isolated IGT have, by definition, fasting plasma glucose levels that are similar to those with no glucose tolerance (NGT). However, following glucose ingestion the plasma glucose concentration rises excessively at all-time points and remains elevated (140–199 mg/dl) after 120 min. On the other hand, in isolated IFG, the fasting plasma glucose is higher (100–125 mg/dl) than in NGT and isolated IGT, and the plasma glucose concentrations at 30–60 min in the OGTT are greater than in both NGT and isolated IGT. Thereafter, the plasma glucose concentration in IFG declines to near-baseline values at 120 min. These two very distinct oral

glucose tolerance curves reflect different pathophysiologic disturbances in glucose homeostasis in isolated IFG and isolated IGT. The plasma glucose curves in people with both IFG and IGT reflect the characteristics of both. Although both isolated IFG and isolated IGT are insulin-resistant states, they differ in their site of insulin resistance. People with isolated IFG predominantly have hepatic insulin resistance and normal muscle insulin sensitivity, whereas individuals with isolated IGT have normal to slightly reduced hepatic insulin sensitivity and moderate to severe muscle insulin resistance. Not surprisingly, individuals with both IFG and IGT manifest both muscle and hepatic insulin resistance (David *et al*., 2007).

The pattern of insulin secretion also differs between IFG and IGT. People with isolated IFG have a decrease in first phase (0–10 min) insulin secretory response to intravenous glucose and a reduced early phase (first 30 min) insulin response to oral glucose. However, the late-phase (60–120 min) plasma insulin response during the OGTT is normal in isolated IFG. Isolated IGT also has a defect in early-phase insulin secretion in response to an oral glucose load and in addition has a severe deficit in late phase insulin secretion. The combination of hepatic insulin resistance and defective insulin secretion in isolated IFG results in excessive fasting hepatic glucose production accounting for fasting hyperglycemia. The impairment in early insulin response in combination with hepatic insulin resistance results in the excessive early rise of plasma glucose in the 1st hour of the OGTT. However, the preservation of late insulin secretion combined with normal muscle insulin sensitivity allows glucose levels to return to the preload value in isolated IFG. In contrast, in isolated IGT the defective late insulin secretion, combined with muscle and hepatic insulin resistance, results in prolonged hyperglycemia after a glucose load (David *et al*., 2007).

1.2.1.6 Gestational Diabetes Mellitus

Gestational diabetes is a condition in which a woman without diabetes develops high blood sugar levels during pregnancy; Gestational diabetes generally results in few symptoms however, it does increase the risk of pre-eclampsia, depression, and requiring a Caesarean section. Babies born to mothers with poorly treated gestational diabetes are at increased risk of being too large, having low blood sugar after birth, and jaundice. If untreated, it can also result in a stillbirth. Long term, children are at higher risk of being overweight and developing type 2 diabetes; gestational diabetes is caused by not enough insulin in the setting of insulin resistance. Risk factors include being overweight, previously having gestational diabetes, a family history of type 2 diabetes, and having polycystic ovarian syndrome. Diagnosis is by blood tests. For those at normal risk, screening is recommended between 24- and 28-weeks' gestation. For those at high risk, testing may occur at the first prenatal visit. Prevention is by maintaining a healthy weight and exercising before pregnancy. Gestational diabetes is treated with a diabetic diet, exercise, and possibly insulin injections. Most women can manage their blood sugar with diet and exercise. Blood sugar testing among those who are affected is often recommended four times a day. Breastfeeding is recommended as soon as possible after birth; gestational diabetes affects 3–9 % of pregnancies, depending on the population studied; it is especially common during the last three months of pregnancy. It affects 1% of those under the age of 20 and 13 % of those over the age of 44. Several ethnic groups including Asians, American Indians, Indigenous Australians, and Pacific Islanders are at higher risk of suffering from gestational diabetes. In 90% of cases, gestational diabetes will resolve after the baby is born. Women, however, are at an increased risk of developing type 2 diabetes. A woman is diagnosed with gestational diabetes when glucose intolerance continues beyond 24 to 28 weeks of gestation (Donovan and McIntyre, 2010).

The two subtypes of gestational diabetes under this classification system are:

Type A1: abnormal oral glucose tolerance test (OGTT), but normal blood glucose levels during fasting and two hours after meals; diet modification is sufficient to control glucose levels.

Type A2: abnormal OGTT compounded by abnormal glucose levels during fasting and/or after meals; additional therapy with insulin or other medications is required. Diabetes which existed prior to pregnancy is also split up into several subtypes under this system;

Type B: onset at age 20 or older and duration of less than 10 years.

Type C: onset at age 10–19 or duration of 10–19 years.

Type D: onset before age 10 or duration greater than 20 years.

Type E: overt diabetes mellitus with calcified pelvic vessels.

Type F: diabetic nephropathy.

Type R: proliferative retinopathy.

Type RF: retinopathy and nephropathy.

Type H: ischemic heart disease.

Type T: prior kidney transplant.

An early age of onset or long-standing disease comes with greater risks, hence the first three subtypes. Two other sets of criteria are available for diagnosis of gestational diabetes, both based on blood-sugar levels; criteria for diagnosis of gestational diabetes, using the 100-gram Glucose

Tolerance Test are fasting has a normal range of 95 mg/dL, 1hour after eating has glucose tolerance test level to be 180 mg/dL, 2 hours after eating has 155 mg/dL, 3 hours has 140 mg/dL. Criteria for diagnosis of gestational diabetes according to National Diabetes Data Group, Fasting 105 mg/dl, 1 hour 190 mg/dl, 2 hours 165 mg/dL, 3 hours 145 mg/dL.

1.2.1.6.1 Pathophysiology of Gestational Diabetes Mellitus

The precise mechanisms underlying gestational diabetes mellitus (GDM) remain unknown. The hallmark of GDM is increased insulin resistance. Pregnancy hormones and other factors are thought to interfere with the action of insulin as it binds to the insulin receptor. The interference probably occurs at the level of the cell signaling pathway beyond the insulin receptor. Since insulin promotes the entry of glucose into most cells, insulin resistance prevents glucose from entering the cells properly. As a result, glucose remains in the bloodstream, where glucose levels rise. More insulin is needed to overcome this resistance; about 1.5–2.5 times more insulin is produced than in a normal pregnancy. Insulin resistance is a normal phenomenon emerging in the second trimester of pregnancy, which in cases of GDM progresses thereafter to levels seen in a non-pregnant person with type-2 diabetes. It is thought to secure glucose supply to the growing fetus. Women with GDM have an insulin resistance that they cannot compensate for with increased production in the β-cells of the pancreas.

Placental hormones, and, to a lesser extent, increased fat deposits during pregnancy, seem to mediate insulin resistance during pregnancy. Cortisol and progesterone are the main culprits, but human placental lactogen, prolactin and estradiol contribute too. Multivariate stepwise regression analysis reveals that, in combination with other placental hormones, leptin, tumor necrosis factor alpha, and resistin are involved in the decrease in insulin sensitivity occurring

during pregnancy, with tumor necrosis factor alpha named as the strongest independent predictor of insulin sensitivity in pregnancy. An inverse correlation with the changes in insulin sensitivity from the time before conception through late gestation accounts for about half of the variance in the decrease in insulin sensitivity during gestation: in other words, low levels or alteration of TNF alpha factors corresponds with a greater chance of, or predisposition to, insulin resistance or sensitivity. It is unclear why some women are unable to balance insulin needs and develop GDM; however, a number of explanations have been given, similar to those in type 2 diabetes: autoimmunity, single gene mutations, obesity, along with other mechanisms (Gabbe, 2012).

Though the clinical presentation of gestational diabetes is well characterized, the biochemical mechanism behind the disease is not well known. One proposed biochemical mechanism involves insulin-producing β-cell adaptation controlled by the HGF/c-MET signaling pathway. β-cell adaption refers to the change that pancreatic islet cells undergo during pregnancy in response to maternal hormones to compensate for the increased physiological needs of mother and baby. These changes in the β-cells cause increased insulin secretion as a result of increased β-cell proliferation. HGF/c-MET has also been implicated in β-cell regeneration, which suggests that HGF/c-MET may help increase β-cell mass to compensate for insulin needs during pregnancy. Recent studies support that loss of HGF/c-MET signaling results in aberrant β-cell adaptation. c-MET is receptor tyrosine kinase (RTK) that is activated by its ligand, hepatocyte growth factor (HGF), and is involved in the activation of several cellular processes. When HGF binds c-MET, the receptor homodimerizes and self-phosphorylates to form an SH2 recognition domain. The downstream pathways activated include common signaling molecules such as RAS

and MAPK, which affect cell motility, cell motility, and cell cycle progression (Organ and Tsao, 2011).

Studies have shown that HGF is an important signaling molecule in stress related situations where more insulin is needed. Pregnancy causes increased insulin resistance and so higher insulin demand. The β-cells must compensate for this by either increasing insulin production or proliferating. If neither of the processes occurs, then markers for gestational diabetes are observed. It has been observed that pregnancy increases HGF levels, showing a correlation that suggests a connection between the signaling pathway and increased insulin needs. In fact, when no signaling is present, gestational diabetes is more likely to occur; the exact mechanism of HGF/c-MET regulated β-cell adaptation is not yet known but there are several hypotheses about how the signaling molecules contribute to insulin levels during pregnancy. c-MET may interact with FoxM1, a molecule important in the cell cycle, as FOXM1 levels decrease when c-MET is not present. Additionally, c-MET may interact with p27 as the protein levels increase with c-MET is not present. Another hypothesis says that c-MET may control β-cell apoptosis because a lack of c-MET causes increases cell death but the signaling mechanisms have not been elucidated. Although the mechanism of HGF/c-MET control of gestational diabetes is not yet well understood, there is a strong correlation between the signaling pathway and the inability to produce an adequate amount of insulin during pregnancy and thus it may be the target for future diabetic therapies (Demirci *et al.*, 2012). Because glucose travels across the placenta (through diffusion facilitated by GLUT1 carrier), which is in the syncytiotrophoblast on both the microvillus and basal membranes, these membranes may be the rate-limiting step in placental glucose transport. There is a two-to-three-fold increase in the expression of syncytiotrophoblas glucose transporters with advancing gestation. Finally, the role of GLUT3/GLUT4 transport

remains speculative. If the untreated gestational diabetes fetus is exposed to consistently higher glucose levels, this leads to increased fetal levels of insulin (insulin itself cannot cross the placenta). The growth stimulating effects of insulin can lead to excessive growth and a large body (macrosomia). After birth, the high glucose environment disappears, leaving these newborns with ongoing high insulin production and susceptibility to low blood glucose levels (hypoglycemia) (Kelly *et al*., 2005)

1.2.1.6.2 Screening Gestational Blood Glucose

1.2.1.6.2.1 Non-challenge blood glucose tests

When a plasma glucose level is found to be higher than 126 mg/dl (7.0 mmol/l) after fasting, or over 200 mg/dl (11.1 mmol/l) on any occasion, and if this is confirmed on a subsequent day, the diagnosis of gestational diabetes mellitus is made, and no further testing is required. These tests are typically performed at the first antenatal visit. They are simple to administer and inexpensive but have a lower test performance compared to the other tests, with moderate sensitivity, low specificity and high false positive rates.

1.2.1.6.2.2 Screening glucose challenge test

The screening glucose challenge test (sometimes called the O'Sullivan test) is performed between 24–28 weeks and can be seen as a simplified version of the oral glucose tolerance test (OGTT). No previous fasting is required for this screening test in contrast to the OGTT. The O'Sullivan test involves drinking a solution containing 50 grams of glucose and measuring blood levels 1 hour later; if the cut-off point is set at 140 mg/dl (7.8 mmol/l), 80% of women with GDM will be detected. If this threshold for further testing is lowered to 130 mg/dl, 90% of GDM

cases will be detected, but there will also be more women who will be subjected to a consequent OGTT unnecessarily.

1.2.1.6.2.3 Oral glucose tolerance test

A standardized oral glucose tolerance test (OGTT) should be done in the morning after an overnight fast of between 8 and 14 hours. During the three previous days the subject must have an unrestricted diet (containing at least 150 g carbohydrate per day) and unlimited physical activity. The subject should remain seated during the test and should not smoke throughout the test.

The test involves drinking a solution containing a certain amount of glucose, usually 75 g or 100 g, and drawing blood to measure glucose levels at the start and on set time intervals thereafter.

The diagnostic criteria from the National Diabetes Data Group (NDDG) have been used most often, but some centers rely on the Carpenter and Coustan criteria, which set the cutoff for normal at lower values. Compared with the NDDG criteria, the Carpenter and Coustan criteria lead to a diagnosis of gestational diabetes in 54 percent more pregnant women, with an increased cost and no compelling evidence of improved perinatal outcomes.

The following are the values which the American Diabetes Association considers to be abnormal during the 100 g of glucose OGTT:

Fasting blood glucose level ≥ 95 mg/dl (5.33 mmol/L)

1 hour blood glucose level ≥ 180 mg/dl (10 mmol/L)

2 hour blood glucose level ≥ 155 mg/dl (8.6 mmol/L)

3 hour blood glucose level ≥ 140 mg/dl (7.8 mmol/L)

An alternative test uses a 75 g glucose load and measures the blood glucose levels before and after 1 and 2 hours, using the same reference values. This test will identify fewer women who are at risk, and there is only a weak concordance (agreement rate) between this test and a 3-hour 100 g test.

The glucose values used to detect gestational diabetes were first determined by O'Sullivan and Mahan (1964) in a retrospective cohort study (using a 100 grams of glucose OGTT) designed to detect risk of developing type 2 diabetes in the future. The values were set using whole blood and required two values reaching or exceeding the value to be positive. Subsequent information led to alterations in O'Sullivan's criteria. When methods for blood glucose determination changed from the use of whole blood to venous plasma samples, the criteria for gestational diabetes mellitus were also changed. Women with gestational diabetes mellitus may have high glucose levels in their urine (glucosuria). Although dipstick testing is widely practiced, it performs poorly, and discontinuing routine dipstick testing has not been shown to cause underdiagnosis where universal screening is performed. Increased glomerular filtration rates during pregnancy contribute to some 50% of women having glucose in their urine on dipstick tests at some point during their pregnancy. The sensitivity of glucosuria for gestational diabetes mellitus in the first two trimesters is only around 10% and the positive predictive value is around 20% (Ritterath *et al.,* 2006).

1.2.1.6.3 Prevention of Gestational Diabetes

A 2015 review found that when done during pregnancy moderate physical exercise is effective for the prevention of gestational diabetes. A 2014 review however did not find a significant

effect. It is uncertain if additional dietary advice interventions help to reduce the risk of gestational diabetes; Diet and physical activity interventions designed to prevent excessive gestational weight gain reduce the rates of gestational diabetes. However, the impact of these interventions varies with the body-mass index of the person as well as with the region in which the studies were performed (David *et al.,* 2007).

It has been suggested that for women who have had gestational diabetes, support between pregnancies may lower their chances of having gestational diabetes again in future pregnancies. This support might include diet and exercise, education, and lifestyle advice; theoretically, smoking cessation may decrease the risk of gestational diabetes among smokers.

1.2.1.6.4 Management of Gestational Diabetes

Treatment of gestational diabetes mellitus with diet and insulin reduces health problems in mother and child. Treatment of gestational diabetes mellitus is also accompanied by more inductions of labor. A repeat oral glucose tolerance test should be carried out 6 weeks after delivery, to confirm the diabetes has disappeared. Afterwards, regular screening for type 2 diabetes is advised. Lifestyle interventions include exercise, diet advice, behavioral interventions, relaxation, self-monitoring glucose, and combined interventions. Women with gestational diabetes who receive lifestyle interventions seem to have less postpartum depression, and were more likely to reach their weight loss targets after giving birth, than women who had no intervention. Their babies are also less likely to be large for their gestational age, and have less percentage of fat when they are born. If a diabetic diet or glycemic index diet, exercise, and oral medication are inadequate to control glucose levels, insulin therapy may become necessary. The development of macrosomia can be evaluated during pregnancy by using sonography.

Women who use insulin, with a history of stillbirth, or with hypertension are managed like women with diabetes (Alwan *et al.,* 2009).

1.2.1.6.5 Lifestyle

Counselling before pregnancy (for example, about preventive folic acid supplements) and multidisciplinary management are important for good pregnancy outcomes. Most women can manage their gestational diabetes mellitus with dietary changes and exercise. Self-monitoring of blood glucose levels can guide therapy. Some women will need anti-diabetic drugs, most commonly insulin therapy. Diet taken needs to provide sufficient calories for pregnancy, typically 2,000 – 2,500 kcal with the exclusion of simple carbohydrates. The main goal of dietary modifications is to avoid peaks in blood sugar levels. This can be done by spreading carbohydrate intake over meals and snacks throughout the day and using slow-release carbohydrate sources known as the Glycemic index Diet. Since insulin resistance is highest in mornings, breakfast carbohydrates need to be restricted more. Ingesting more fiber in foods with whole grains, or fruit and vegetables can also reduce the risk of gestational diabetes. There is not enough evidence to indicate if one type of dietary advice is better than another (Han *et al*., 2017).

Regular moderately intense physical exercise is advised, although there is no consensus on the specific structure of exercise programs for gestational diabetes mellitus. Pregnant women who exercise have lower blood sugar levels when fasting and after meals compared to those who do not exercise. It is not clear which form of exercise is best when pregnant. Self-monitoring can be accomplished using a handheld capillary glucose dosage system. Compliance with these glucometer systems can be low (Han *et al*., 2017).

1.2.1.6.6 Medication

If monitoring reveals failing control of glucose levels with these measures, or if there is evidence of complications like excessive fetal growth, treatment with insulin might be necessary. This is most commonly fast-acting insulin given just before eating to blunt glucose rises after meals. Care needs to be taken to avoid low blood sugar levels due to excessive insulin. Insulin therapy can be normal or very tight; more injections can result in better control but requires more effort, and there is no consensus that it has large benefits. A 2016 Cochrane review concluded that quality evidence is not yet available to determine the best blood sugar range for improving health for pregnant women with gestational diabetes mellitus and their babies. There is some evidence that certain medications by mouth might be safe in pregnancy, or at least, are less dangerous to the developing fetus than poorly controlled diabetes. When comparing which diabetes tablets (medication by mouth) work best and are safest, there is not enough quality research to support one medication over another. The medication metformin is better than glyburide. If blood glucose cannot be adequately controlled with a single agent, the combination of metformin and insulin may be better than insulin alone. Another review found good short-term safety for both the mother and baby with metformin but unclear long term safety (Butalia *et al*., 2017).

People may prefer metformin by mouth to insulin injections. Treatment of polycystic ovarian syndrome with metformin during pregnancy has been noted to decrease gestational diabetes mellitus levels Almost half of the women did not reach sufficient control with metformin alone and needed supplemental therapy with insulin; compared to those treated with insulin alone, they required less insulin, and they gained less weight. With no long-term studies into children of women treated with the drug, there remains a possibility of long-term complications from

metformin therapy. Babies born to women treated with metformin have been found to develop less visceral fat, making them less prone to insulin resistance in later life (Sivalingam *et al*., 2014).

1.2.1.6.7 Prognosis

Gestational diabetes generally resolves once the baby is born. Based on different studies, the chances of developing gestational diabetes mellitus in a second pregnancy, if a woman had gestational diabetes mellitus in her first pregnancy, the chances of having it again are between 30 and 84%, depending on ethnic background. A second pregnancy within one year of the previous pregnancy has a large likelihood of gestational diabetes mellitus recurrence women diagnosed with gestational diabetes have an increased risk of developing diabetes mellitus in the future. The risk is highest in women who needed insulin treatment, had antibodies associated with diabetes (such as antibodies against glutamate decarboxylase, islet cell antibodies and/or insulinoma antigen-2), women with more than two previous pregnancies, and women who were obese; women requiring insulin to manage gestational diabetes have a 50% risk of developing diabetes within the next five years. Depending on the population studied, the diagnostic criteria and the length of follow-up, the risk can vary enormously. The risk appears to be highest in the first 5 years, reaching a plateau thereafter. One of the longest studies followed a group of women from Boston, Massachusetts; half of them developed diabetes after 6 years, and more than 70% had diabetes after 28 years. In a retrospective study in Navajo women, the risk of diabetes after gestational diabetes mellitus was estimated to be 50 to 70 % after 11 years (Steinhart *et al.,* 1997).

Another study found a risk of diabetes after gestational diabetes mellitus of more than 25% after 15 years. In populations with a low risk for type 2 diabetes, in lean subjects and in women with autoantibodies, there is a higher rate of women developing type 1 diabetes. Children of women with gestational diabetes mellitus have an increased risk for childhood and adult obesity and an increased risk of glucose intolerance and type 2 diabetes later in life. This risk relates to increased maternal glucose values. It is currently unclear how much genetic susceptibility and environmental factors contribute to this risk, and whether treatment of gestational diabetes mellitus can influence this outcome. Relative benefits and harms of different oral anti-diabetic medications are not yet well understood as of 2017. There are scarce statistical data on the risk of other conditions in women with gestational diabetes mellitus; in the Jerusalem Perinatal study, 410 out of 37,962 women were reported to have gestational diabetes mellitus, and there was a tendency towards more breast and pancreatic cancer, but more research is needed to confirm this finding (Perrin *et al.,* 2007).

1.2.1.6.8 Complication

Gestational diabetes mellitus poses a risk to mother and child. This risk is largely related to uncontrolled blood glucose levels and its consequences. The risk increases with higher blood glucose levels. Treatment resulting in better control of these levels can reduce some of the risks of gestational diabetes mellitus considerably.

The two main risks gestational diabetes mellitus imposes on the baby are growth abnormalities and chemical imbalances after birth, which may require admission to a neonatal intensive care unit. Infants born to mothers with gestational diabetes mellitus are at risk of being both large for gestational age (macrosomic) in unmanaged gestational diabetes mellitus, and small for

gestational age and intrauterine growth retardation in managed gestational diabetes mellitus. Macrosomia in turn increases the risk of instrumental deliveries (e.g. forceps, ventouse and caesarean section) or problems during vaginal delivery (such as shoulder dystocia). Macrosomia may affect 12% of normal women compared to 20% of women with gestational diabetes mellitus (GDM). However, the evidence for each of these complications is not equally strong; in the hyperglycemia and adverse Pregnancy outcome (HAPO) study for example, there was an increased risk for babies to be large but not small for gestational age in women with uncontrolled GDM. Research into complications for GDM is difficult because of the many confounding factors (such as obesity). Labelling a woman as having GDM may increase the risk of having an unnecessary caesarean section (Jovanovic et *al.,* 1997).

Neonates born from women with consistently high blood sugar levels are also at an increased risk of low blood glucose (hypoglycemia), jaundice, high red blood cell mass (polycythemia) and low blood calcium (hypocalcemia) and magnesium (hypomagnesemia). Untreated GDM also interferes with maturation, causing immature babies that is prone to respiratory distress syndrome due to incomplete lung maturation and impaired surfactant synthesis. Unlike pre-gestational diabetes, gestational diabetes has not been clearly shown to be an independent risk factor for birth defects. Birth defects usually originate sometime during the first trimester (before the 13th week) of pregnancy, whereas GDM gradually develops and is least pronounced during the first and early second trimester. Studies have shown that the offspring of women with GDM are at a higher risk for congenital malformations. A large case-control study found that gestational diabetes was linked with a limited group of birth defects, and that this association was generally limited to women with a higher body mass index ($\geq$25 kg/m^2). It is difficult to make

sure that this is not partially due to the inclusion of women with pre-existent type 2 diabetes who were not diagnosed before pregnancy. Because of conflicting studies, it is unclear now whether women with GDM have a higher risk of preeclampsia. In the HAPO study, the risk of preeclampsia was between 13 % and 37 % higher, although not all possible confounding factors were corrected (Metzger *et al*., 2002).

1.3.2 Streptozotocin

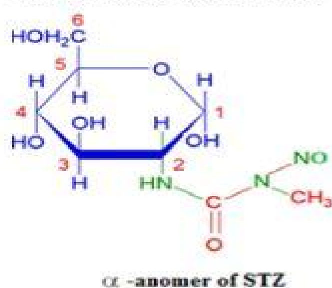
Streptozotocin (STZ) is a permanent diabetes inducing drug. It is synthesized by a strain of the soil microbe *Streptomyces achromogenes* (gram positive bacterium) with broad spectrum of antibacterial properties. Streptozotocin is an unusual aminoglycoside containing a nitrosoamino group discovered in 1959 as an antibiotic, now marketed as a generic drug. The nitrosoamino group enables the metabolite to act as a nitric oxide (NO) donor. Nitric oxide 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; streptozotocin (STZ) 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 streptozotocin 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 β-cells transports streptozotocin 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 streptozotocin. Streptozotocin is widely used to induce both insulin-dependent (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM).

Streptozotocin is an antibiotic and antitumor agent, induces diabetes mellitus via reduction of nicotinamide adenine dinucleotide (NAD^+) in pancreatic β-cells in vivo (Busineni *et al*., 2015).

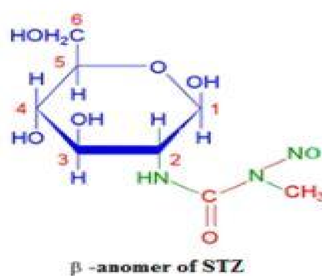
1.3.2.1 Structural features of streptozotocin

Streptozotocin (2-deoxy-2-[3-methyl-3-nitrosourea] 1-D-glucopyranose) occurs in two anomeric forms, α and β, 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 $C_8H_{15}N_3O_7$. Streptozotocin molecular structure is similar to that of 2-deoxy-D-glucose with a replacement at C_2 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).

a. ANOMERIC FORMS OF STREPTOZOTOCIN

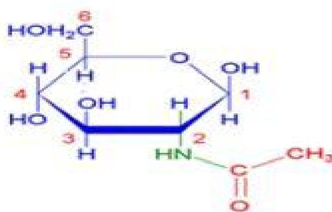


α -anomer of STZ



β -anomer of STZ

b. N-ACETYL GLUCOSAMINE



c. METHYLNITROSOUREA

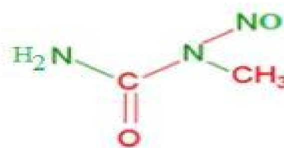


Figure 1.2: Chemical structure of STZ (Busineni *et al.*, 2015)**.** A schematic diagram representing - a) α and β Anomeric forms of STZb) structural analog of STZ - N-acetyl glucosamine c) cytotoxic moiety of STZ- N-methyl-Nnitrosourea

1.2.2.2 Solubility and Stability

In case of solubility, streptozotocin is highly soluble in water, ketones and lower alcohols, but slightly soluble in polar organic solvents. This product dissolves in water at 50 mg/mL to give a light-yellow solution, from clear to slightly hazy. Aqueous solutions of streptozotocin rapidly undergo mutarotation to an equilibrium mixture of alpha- and beta-anomers. Maximum stability of streptozotocin solution is at pH 4, with stability decreasing rapidly at higher or lower pH. Freshly prepared solutions are clear and have a light straw color. On standing, they take on a yellow to brown color and effervescent, indicating decomposition. streptozotocin can be stored at 4 ^{o}C for short term, but long term storage needs -20 ^{o}C because it is stable at this temperature for at least 2 years The streptozotocin solution (in citrate or acetate buffer, pH 4.5) should be administered immediately but not later than 15 to 20 min after dissolving, as recommended by different research consortia. Solutions should be prepared just before use, since the product is unstable. Streptozotocin is dissolved in Phosphate buffer saline (PBS) instead of acidic citrate buffer, which allegedly rapidly inactivates the drug. Solutions of Streptozotocin will spontaneously give off NO gas at room temperature. This NO release is slowed, but not completely stopped even at -80 °C, and the rate of NO release is also impacted by the solvent used (for example, dissolving Streptozotocin in buffers that contain sodium speeds up NO release). Since it does not seem to be possible to stop completely the NO release when Streptozotocin is in solution, and the NO donor function is critical to most experimental applications, so it is recommended that to make STZ solutions only immediately before use.

Streptozotocin is cytotoxic to pancreatic β-cells and its effects can be seen within seventy-two hours after administration depending on the dose administered (Busineni *et al*., 2015).

1.2.2.3 Route of administration and Dose Schedule of streptozotocin

A wide variety of dose schedules and routes of administration have been reported in inducing diabetes in rats with streptozotocin. Streptozotocin is most delivered by one of two routes, intraperitoneal (IP) or intravenous (IV), although other methods including subcutaneous, intracardiac, and intramuscular delivery have been used in rodents. Although intraperitoneal offers a quick and easy method of administration, especially for studies involving multiple doses of the drug, accidental delivery into the bowel or sub-dermal space may result in increased morbidity or decrease in diabetogenic effect. Additionally, other studies have reported that IV administration of streptozotocin produces a more stable and reproducible model of diabetes than IP administration; the two most common protocols of dose schedule are intraperitoneal injection of a single high dose or multiple low doses. The frequently used single intravenous dose of streptozotocin in adult rats to induce IDDM is between 50 and 55 mg/kg body weight, but higher doses are also used. Streptozotocin is also efficacious after intraperitoneal administration of a similar or higher dose, but single dose below 40 mg/kg body weight may be ineffective (Busineni *et al*., 2015).

1.2.4 *Justicia carnea*

Justicia carnea is an upright, evergreen shrub, 3 to 7 feet tall and wide, with large, 6-inch-long, dark green leaves and upwardly-facing plumes of tubular, slightly fragrant flower clusters in rose-purple, red, yellow, orange, apricot, or white (depending on the selection), which appear from early summer until fall (Otuokere *et al.,* 2016). The plant cycles through 2 to 4 major

flowering periods during the year, with few flowers in between. Many stems appear from the center of the plant and rise straight up for several feet before branching. The rapid growth and evergreen nature make *J. carnea* ideal for use as a foundation or mass planting, used in various containers, or may even be trained into an attractive espalier. Use it in front of a green shrubbery border to create a color accent throughout the warm months (Udedi *et al.,* 2020).

1.2.4.1 General Information

Scientific name: *Justicia carnea*

Pronunciation: juss-TISH-ee-uh KAR-nee-uh

Common name(s): Jacobinia, Flamingo Plant

Family: *Acanthaceae*

Plant type: perennial; herbaceous

USDA hardiness zones: 8B through 11

Planting month for zone 8: year round

Planting month for zone 9: year round

Planting month for zone 10 and 11: year round

Origin: not native to North America

1.2.4.2 Description

Height: 3 to 6 feet

Spread: 2 to 3 feet

Plant habit: upright; spreading

Plant density: open **Fruit shape:** pod or pod-like

Growth rate: slow **Fruit length:** less than .5 inch

Texture: coarse **Fruit cover:** dry or hard

1.2.4.3 Foliage

Leaf arrangement: opposite/subopposite

Leaf type: simple

Leaf margin: undulate **Trunk/bark/branches:** not particularly showy; typically

Leaf shape: elliptic (oval)

Leaf venation: pinnate

Leaf type and persistence: deciduous

Leaf blade length: 8 to 12 inches

Leaf color: green

Fall color: no fall color change

Fall characteristic: not showy

1.2.4.4 Flower

Flower color: white; pink; apricot; yellow; lavender

Flower characteristic: flowers periodically throughout

the year

1.2.4.5 Fruit

Fruit color: brown

Fruit characteristic: inconspicuous and not showy

Trunk and Branches: multi-trunked or clumping stems

Current year stem/twig color: green

Current year stem/twig thickness: very thick

1.2.4.6 Culture

Light requirement: plant grows in the shade

Soil tolerances: acidic; slightly alkaline; sand; loam; clay

1.2.4.7 Drought tolerance

Soil salt tolerances: poor

Plant spacing: 24 to 36 inches

Roots: usually not a problem

Winter interest: no special winter interest

Outstanding plant: plant has outstanding ornamental features and could be planted more

Invasive potential: not known to be invasive

Pest resistance: long-term health usually not affected by pests

1.2.4.8 Use and Management

Rain and irrigation weigh the foliage down and cause the branches to spread out, sometimes touching the ground. This limits height growth and opens up the plant to allow light to enter the interior, stimulating growth from the lower stem and inner branches (Corrêa and Alcântara, 2012). It has a place in almost every garden in USDA hardiness zones 8b through 11. *Justicia carnea* is grown on rich, well-drained soil which receives ample moisture in a location shaded from full-day sun. Although tender to frost, plants will grow back quickly in springtime in USDA hardiness zones 8 and 9. The only necessary pruning during the growing season should be limited to removal of dead flower heads or occasional pinching to stimulate branching (Corrêa and Alcântara, 2012). Since flowers form on new growth, prune early in the spring so flower display is not reduced. This increases the number of branches and flowers and creates an attractive, full plant. Older plants benefit from rejuvenation type pruning where most of the taller

stems are cut back to a node close to the ground. If this is performed each year, plants should last for many years. Propagation is by cuttings. Nematodes, spider mites and caterpillars occasionally chew portions of the foliage, but these will not kill the plant (Daniel, 2020).

Figure 1.1: Leaves of *J. carnea* (Manokari *et al.,* 2019).

1.5 OXIDATIVE STRESS

Oxidative stress is a phenomenon caused by an imbalance between production and accumulation of oxygen reactive species (ROS) in cells and tissues and the ability of a biological system to detoxify these reactive products. ROS can play, and in fact they do it, several physiological roles (i.e., cell signaling), and they are normally generated as by-products of oxygen metabolism; despite this, environmental stressors (i.e., UV, ionizing radiations, pollutants, and heavy metals) and xenobiotics (i.e., antiblastic drugs) contribute to greatly increase ROS production, therefore causing the imbalance that leads to cell and tissue damage (oxidative stress). Several antioxidants

have been exploited in recent years for their actual or supposed beneficial effect against oxidative stress, such as vitamin E, flavonoids, and polyphenols (Gabriele *et al.,* 2017).

Superoxide radicals (O2•−), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen (1O2) are commonly defined reactive oxygen species (ROS); they are generated as metabolic by-products by biological systems. Processes, like protein phosphorylation, activation of several transcriptional factors, apoptosis, immunity, and differentiation, are all dependent on a proper ROS production and presence inside cells that need to be kept at a low level. When ROS production increases, they start showing harmful effects on important cellular structures like proteins, lipids, and nucleic acids. A large body of evidences shows that oxidative stress can be responsible, with different degrees of importance, in the onset and/or progression of several diseases (i.e., cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular diseases) (Taniyama and Griendling, 2003).

ROS are mainly produced by mitochondria, during both physiological and pathological conditions, that is, O2•− can be formed by cellular respiration, by lipoxygenases (LOX) and cyclooxygenases (COX) during the arachidonic acid metabolism, and by endothelial and inflammatory cells. Despite the fact that these organelles have an intrinsic ROS scavenging capacity, it is worth to note that this is not enough to address the cellular need to clear the amount of ROS produced by mitochondria. Cells deploy an antioxidant defensive system based mainly on enzymatic components, such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), to protect themselves free ROS-induced cellular damage (Gabriele *et al.,* 2017).

1.5.1 Oxidants and Free Radicals Production

Reactive Oxygen Species (ROS) production basically relies on enzymatic and non-enzymatic reactions. Enzymatic reactions able to generate ROS are those involved in respiratory chain, prostaglandin synthesis, phagocytosis, and cytochrome P450 system. Superoxide radical is generated by NADPH oxidase, xanthine oxidase, and peroxidases. Once formed, it is involved in several reactions that in turn generate hydrogen peroxide, hydroxyl radical (OH•), peroxynitrite (ONOO−), hypochlorous acid (HOCl), and so on. H2O2 (a non-radical) is produced by multiple oxidase enzymes, that is, amino acid oxidase and xanthine oxidase. Hydroxyl radical (OH•), the most reactive among all the free radical species in vivo, is generated by reaction of O2•− with H2O2, with Fe2+ or Cu+ as a reaction catalyst (Fenton reaction). Nitric oxide radical (NO•), which plays some important physiological roles, is synthesized free *Radicals* arginine-to-citrulline oxidation by nitric oxide synthase (NOS) (Deponte, 2013). Even non enzymatic reactions can be responsible for *Free* radical production, that is, when oxygen reacts with organic compounds or when cells are exposed to ionizing radiations. Non enzymatic free radical production can occur as well during mitochondrial respiration (Taniyama and Griendling, 2003).

Free radicals are generated from both endogenous and exogenous sources. Immune cell activation, inflammation, ischemia, infection, cancer, excessive exercise, mental stress, and aging are all responsible for endogenous free radical production. Exogenous free radicals production can occur as a result on exposure to environmental pollutants, heavy metals (Cd, Hg, Pb, Fe, and As), certain drugs (cyclosporine, tacrolimus, gentamycin, and bleomycin), chemical solvents, cooking (smoked meat, used oil, and fat), cigarette smoke, alcohol, and radiations. When these exogenous compounds penetrate the body, they are degraded or metabolized, and free radicals are generated as by-products (Valko *et al.,* 2005).

1.5.2 Detrimental Effects of Free Radicals on Human Health

If in excess, *Free* radicals and oxidants give rise to a phenomenon known as oxidative stress; this is a harmful process that can negatively affect several cellular structures, such as membranes, lipids, proteins, lipoproteins, and deoxyribonucleic acid (DNA) (Young and Woodside, 2001). Oxidative stress emerges when an imbalance exists between *Free* radical formation and the capability of cells to clear them. For instance, an excess of hydroxyl radical and peroxynitrite can cause lipid peroxidation, thus damaging cell membranes and lipoproteins. This in turn will lead to malondialdehyde (MDA) and conjugated diene compound formation, which are known to be cytotoxic as well as mutagenic. Being a radical chain reaction, lipid peroxidation spreads very quickly affecting a large amount of lipid molecules. Proteins may as well being damaged by oxidative stress, undergoing to conformational modifications that could determine a loss, or an impairment, of their enzymatic activity. Even DNA is prone to oxidative stress-related lesions, the most representative of which is the 8-oxo-2′-deoxyguanosine (8-OHdG) formation; this is a particularly pernicious DNA lesion, which can be responsible for both mutagenesis. If not strictly controlled, oxidative stress can be responsible for the induction of several diseases, both chronic and degenerative, as well as speeding up body aging process and cause acute pathologies (that is, trauma and stroke) (Taniyama and Griendling, 2003).

1.6 Antioxidant Enzymes

In living cell, two antioxidant defense system are present against *Free* radical damage. The first line of defense includes antioxidant enzymes (such as superoxide dismutase, catalase, GSH peroxidase), whereas the second defence system includes low molecular non-enzymatic antioxidants (thioredoxin, GSH, vitamins A, C, E, lycopene, lutein, quercetin, amongst others). These antioxidants inhibit the formation of free radicals by breaking the chain reaction or can

reduce the concentration of free radicals by donating hydrogen and an electron. They also act as peroxide decomposer (vitamin E), enzyme inhibitor, singlet oxygen quencher (vitamin E), synergist and metal chelating agents (transferrin). To provide maximum intracellular protection, antioxidants are strategically compartmentalized throughout the cell. So that free radicals is produced intracellular and extracellular during metabolism, both enzymatic and non-enzymatic antioxidants are able to detoxify free radicalss (Kumar *et al.,* 2011).

Certain antioxidant enzymes (superoxide dismutase, catalase and GSH) are produced within the body. Other antioxidant agents are found in foods, such as green leafy vegetables, and it is believed that diets rich in antioxidant (such as b-carotene and vitamins A, C and E) are beneficial to human health (Halliwell and Gutteridge, 1989). Therefore, antioxidant naturally present in body or supplied in the form of diet (phytonutrients) plays an important role to control various diseases resulting from oxidative stress. In recent years, researchers have been researching the relationship between antioxidants and prevention of some diseases, such as cardiovascular disease and cancer (Kubola and Siriamornpun, 2008).

As soon as these free radicals are generated in the body, they are trapped by antioxidant present in extracellular and intracellular defense system. If the generation is much more than the concentration of antioxidants then oxidative stress arises. As a result of oxidative stress, arthritis in joints, emphysema and bronchitis in lungs, atherosclerosis or heart disease in the blood vessels, peptic ulcer in the stomach, ageing and wrinkling in the skin are caused. In the nucleus, it also alters the sequence of nucleotide base pair, strand break, amongst others, in the DNA resulting in transformed and mutated DNA. Mutated DNA will produce diseases like cancer, leukaemia and lymphoma (Prakash *et al.,* 2012).

CHAPTER TWO

MATERIALS AND METHOD

2.1 Materials and Apparatus

- *Justicia leaves*
- Wistar rats
- pH meter
- pipette
- Separating funnel
- Retort stand
- Glass jar
- Measuring cylinder
- Lancet mortar and pestle
- Spatula
- Oral gavage
- Spectrophotometer
- Centrifuge
- Aluminum foil
- Glass rod
- Measuring cylinder
- Gloves
- Cotton wool
- Nose mask
- Beakers
- Test tube
- Dissecting material
- Blade
- Scissors
- Syringe
- Cuvette
- Micropipette
- Glucometer

- Test strip
- EDTA container
- Lithium heparin container
- Plain containers
- Masking tape
- Universal bottle
- Test tubes
- Test tubes racks
- Dissecting set
- Micro- centrifuge tube

2.2 Chemicals and Reagents

All chemicals and reagents were of analytical grade and were obtained from standard commercial suppliers. They include;

- Ethyl-Acetate
- Methanol
- Streptozotocin
- Sodium citrate
- Citric acid
- Ethanol
- Potassium hydroxide
- Distilled water
- Methylated spirit
- Picric acid
- Randox Urea Kit
- Randox potassium kit
- Randox chloride kit
- Randox sodium kit
- Randox bicarbonate kit

2.3 Equipment

- Electrical weighing balance 2)
- Freeze drying machine
- Spectrophtometer
- Water bath
- Oven
- Glucometer
- Centrifuge
- Muffle furnace
- Refrigerator

2.4 Plant Material

The leaves of *Justicia carnea* were obtained from a botanical garden along Benin-Lagos express road, Ugbowo, Benin City, Edo State, Nigeria. The plant was identified and confirmed at the Department of Plant Biology and Biotechnology (PBB) University of Benin, Benin City, Edo State Nigeria.

2.5 Preparation of Plant Sample

The fresh leaves were thoroughly washed under running tap, air dried under shade, crushed using a mechanical grinder into fine powder form.

Extraction was carried out by maceration in which the Powdered sample (1000 g) was soaked in absolute methanol (3000 mL) with constant stirring. After 72 hours (3 days) of soaking, the wine (dark purplish red) colored filtrate was filtered using a double cheese cloth, concentrated under reduced pressure using freeze dryer (Triggers laboratory, University of Benin), and subsequently

water bath at 40 °C to obtain a slurry (dark purple) extract termed *Justicia carnea* methanol extract (JCME).

2.6 Experimental Animal

A total of thirty-six (36) male Wistar rats weighing between 150 and 200 g were used in this study. These animals were obtained from the animal house of the Department of Biochemistry, University of Benin, Benin City, Edo State, Nigeria .The animals were randomly distributed into cages and allowed to acclimatize for 2 weeks in a well-ventilated room under natural lighting condition. Throughout the period of acclimatization, the animals were properly fed daily and their environment cleaned up regularly. Also, at every feeding, the plates and water containers were properly washed and rinsed with clean water. Experimental animal care was in accordance with standard protocol (the Principle of Laboratory Animal Care manual guide of National Institute of Health as approved by the Institution Research Ethics committee.

2.7 Toxicity Study

Oral acute toxicity study on *Justicia carnea* methanol extract (JCME) was carried out according to Lorke method using a total of twelve (12) albino rats in two phases. In phase I, rats were randomized into 3 groups of 3 rats per group. Rats in the 3 different groups received 10, 100 and 1000 mg/kg body weight of JCME orally using oral gavage. The animals were initially examined for signs of toxicity after 60 min of administration and further observed for a period of 24 h. The absence of mortality in phase I necessitated the second phase. In phase two, three rats were separated into 3 groups of one rat per group. Each of the rats received 1500, 2900 and 5000 mg/kg body weight of JCME respectively and examined within 24 h for manifestation of toxicity. Rats were further observed for extended hours of 48 h to see if mortality would occur.

2.8 Study Design

Six (6) rats each were randomly selected, and placed in separate cages and labeled as group I to 6. Each rat was weighed singly across the six cages and recorded appropriately, and were administered intraperitoneally with 120 mg/kg body weight of STZ dissolved in ice-cold normal saline. This was allowed to stay for 48 hours after which the glucose level was checked again and recorded as post-induction blood glucose level. Animas with blood glucose level above 200 mg/dL were taken as diabetic. Thus:

Group 1: normal control group (non-diabetic rat)

Group 2: diabetic rats untreated

Group 3: diabetic rats treated with standard anti-diabetic drug (methformin)

Group 4: diabetic rats + 100mg/kg of methanol extract of *Justicia carnea*

Group 5: diabetic rats + 200mg/kg of methanol extract of *Justicia carnea*

Group 6: diabetic rats + 500mg/kg of methanol extract of *Justicia carnea*

2.9 Determination of Body Weight

The weights of the rats were checked before and after induction of diabetes as well as during the course of the treatment on weekly basis using an electronic weighing balance.

2.10 Administration

The process of administration and treatment of the *Justicia carnea* methanol extract and the methformin continued until 21days. The extracts and methformin were administered orally with the aid of a gavage.

2.11 Animal Sacrifice

At the end of the 21st day of administration, the rats were allowed to fast overnight and sacrificed the following morning by anesthetizing each rat in a closed chamber containing drops of chloroform. Each rat was dissected and blood samples were collected by cardiac puncture using 5 mL into tubes containing ethylene diamine tetra acetic acid (EDTA) for hematological analysis. The liver, pancreas and kidneys were excised and rinsed immediately in a normal saline (0.9% Nacl) to remove blood stain, dried between layers of Whatman filter paper and weighed appropriately using a sensitive weighing balance. Section of livers, pancreas and kidneys were excised and fixed in 10% formalin for histopathology examination.

2.12 Homogenization

The excised kidney was grinded individually in a normal saline (0.9% NACL) using laboratory mortar and pestle, the solution was then added in 5ml sterile tube and labeled appropriately. The tube containing the sample was then placed in a centrifuge and centrifuged for 10minutes. The supernatant where then obtained for further analytical process.

2.13 Determination of Catalase (CAT) Activity

Catalase (CAT) activity was estimated by the method described by Cohen *et al.,* (1970).

Reagents

Hydrogen peroxidase (H_2O_2)

Suphuric acid (6M) H_2SO_4

Preparation of reagents

0.01M $KMnO_4$ was prepared by distilling 0.158g of $KMnO_4$ in 100ml of distilled water. Phosphate buffer (pH 7.4) 0.426g of $NaHPO_4$ and 0.240g of NaH_2PO_4 was weighed and

dissolved in 100ml of distilled water. 6M H_2SO_4 and 32.3ml of conc. H_2SO_4 was added to 66.7ml of distilled water.

Procedure

To an unknown volume of plasma (0.5ml), 5.0ml of H_2O_2 was added. This was mixed by inversion and allowed to stand for 30min. The reaction was stopped by adding 1.5ml of 6M H_2SO_4 and 7ml of 0.01M $KMnO_4$. These were mixed by inversion and allowed to stand for 10min. The absorbance was read at 480nm within 30-60 seconds against distilled water. The enzyme blank was run simultaneously with 1.0ml of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as µmoles of H_2O_2 decomposed/min/mg/protein.

Calculation:

$$\text{Activity} = \frac{OD/min \times V}{M \times V \times L \times Y}$$

Where OD = Absorbance

L= Light path

V= Total volume of reaction sample

M= Molar coefficient of H_2O_2 (40/m/cm)

V= Volume of sample

Y= mg protein in the sample

2.14 Estimation of Superoxide Dismutase (SOD) Activity

This was determined according to the methods of Masra and Fridorich (1972)

Principle

Adrenaline undergoes auto oxidation rapidly to adrenochrome whose concentration can be determined at 420nm with the aid of a spectrophotometer. The auto oxidation of adrenaline depends on the presence of superanions.

Superoxide dismutase inhibits the auto-oxdiation of adrenaline by catalysing the breakdown of superoxide anion. The degree of inhibition reflects the activity of SOD which is determined at 420nm.

Reagent and preparation

Carbonate buffer (0.05M) pH 10.2: This was prepared by dissolving 0.2014g of Na_2CO_3, 0.2604g $NaHCO_3$ and 0.0372g of EDTA in 100ml of distilled water. The pH was adjusted to 10.2 using Sodium hydroxide.

Hydrochloric acid (0.005M): This was prepared by adding 0.044 concentration of HCL to 99.96mls of distilled water.

Adrenaline solution (0.3mM): This was prepared by dissolving 0.01098g of adrenaline in 100mls of 0.005M HCL solution.

Plasma volume of 100ml was mixed with 125ml of carbonate buffer and 150ml of adrenaline solution. 100ml of distilled water was mixed with 1.25ml of carbonate buffer as reference sample. These were mixed and absorbance read at 420nm.

These were mixed and read at 420nm

$$\%\text{inhibition} = \frac{(\text{O.D test} - \text{ODref}) \times 100}{\text{OD test}}$$

Enzyme concentration can thus be calculated

$$\text{Unit/mg protein} = \% \frac{\text{inhibition}}{50 \times Y}$$

Where Y = mg of protein in the volume of sample used

2.15 Estimation of Activity of Gluthathione Peroxidase (GPx)

This was determined according to Nyman (1959)

Principle

This is based on the oxidation of pyrogallol to purpurogallin by peroxidase activity, resulting to a deep brown color disposition, read at 420nm.

Reagent and preparation

Pyrogallol (20mM): 0.2552g of pyrogallol was dissolved in 100mls of distilled water.

Procedures

To an aliquot of plasma (0.2ml), 2.5ml of phosphate buffer, 2.5ml of H_2O_2, 1.5ml of distilled water and 2.5ml of pyrogallol was added.

The reaction was allowed to stand for 30mins at room temperature. A deep brown color was formed which was read at 480nm.

Calculations

$$\text{Activity} = \frac{\text{OD/min} \times \text{vt} \times \text{Df}}{\text{E} \times \text{Vs} \times \text{Y}}$$

OD= Absorbance of test

Vt= Total volume of reaction mixture

Df= Diution factor = 1

E= Molar extinction co-efficient (12/m/cm)

Vs= Volume of sample

Y= mg of protein used

2.16 Determination of Malondialdehyde (MDA) Concentration

Malonaldehyde was determined using the thiobarbituric acid assay (Guttridge and Wilkins, 1982).

Principle

Malonaldehyde which is a product of lipid peroxidation react with thiobabituric acid (TBA) to give a red species.

Procedure

A volume of plasma (1.0ml) was added to 2.0ml of TCA-TBA-HCL and mixed thoroughly. The solution was heated for 15mins in a boiling water bath. After cooling, the flocculent precipitate was removed by centrifuged at 1000g for 10min. The absorbance was determined using the formula;

$$\text{MDA (mol/mg protein)} = A \times \frac{V \times 100}{M \times V \times Y}$$

A= Absorbance

V= Total volume of reaction mixture

M= Molar extinction coefficient

V= volume of the sample

Y= mg protein

2.17 Statistics

Data are presented as mean ± standard error of mean (SEM). The SPSS software was deployed for the statistical analysis.

CHAPTER THREE

RESULTS

Acute Toxicity of Methanol Extract *J. carnea* Leaves

At the highest dose of 5000 mg/kg bwt, methanol extract of *J. carnea* did not produce any mortality in the rats (Table 3.1).

Table 3.1: Results of Acute Toxicity Study

Phase 1

Dose(mg/kg bwt)	**Mortality**
10	**0/3**
100	**0/3**
1000	**0/3**

Phase 2

1600	**0/3**
2900	**0/3**
5000	**0/3**

Effect of *Justicia Carnea* on Glucose Concentration of Streptozotocin Induced Diabetic Rats

Graded doses of methanol extract of *J. carnea* leaves significantly reduced the blood glucose concentration of streptozotocin-induced diabetic rats ($p < 0.05$) (Table 3.2).

Table 3.2: Blood Glucose Concentration of Diabetic Rats

Group			Week		
	Initial	1	2	3	4
Control	76.00±4.15[a]	_	103.67±2.53[a]	92.33±2.37[a]	65.5±3.42[a]
Diabetic Control	94.67±14.45[a]	305.00±27.40[a]	475.00±41.02[b]	368.00±54.08[b]	369.33±28.01[b]
Metformin	62.20±3.88[a]	310.80±56.81[a]	286.40±55.00[c]	204.60±51.17[c]	168.00±76.00[c]
100 mg of Extract	58.80±4.11[a]	283.20±50.71[a]	223.00±58.15[c]	222.00±59.05[c]	171.20±67.12[c]
200 mg of Extract	56.80±1.65[a]	370.20±49.42[a]	321.75±53.10[c]	295±112.155[d]	222.33±62.22[c]
500 mg of Extract	59.17±6.32[a]	297.67±43.31[a]	240.20±70.83[c]	190.00±55.08[c]	53.00±12[a]

Values are expressed as Mean ± SEM.

Values with different superscript in a column are significant at $p < 0.05$

Effect of *Justicia Carnea* on Body Weight of Streptozotocin-Induced Diabetic Rats

The greatest weight increase was observed in the control group when compared to the other groups ($p < 0.05$) (Table 3.3).

Table 3.3: Weight of Rats in the Different Groups

Groups	Initial Body Weight (g) $\times 10^{-3}$	Final Body Weight (g) $\times 10^{-3}$	Change in Body Weight (g) $\times 10^{-3}$
Control	109.53±3.13	192.80±14.34	83.28±14.49
Diabetic Control	133.75±14.41	212.11±17.24	78.36±9.15
Metformin	118.33±5.23	178.58±11.19	66.20±9.74
100 mg of Extract	118.362±12.64	176.67±18.24	58.31±9.52

200 mg of Extract	112.04±4.43	153.99±5.54	41.95±1.45
500 mg of Extract	125.81±6.59	211.08±15.88	85.27±9.29

Values are expressed as Mean ± SEM.

Values with different superscript in a column are significant at $p < 0.05$

Effect of *Justicia carnea* Extract on Antioxidant Status in Streptozotocin-Induced Diabetic Rat

The plant extract significantly increased the activities of catalase, SOD, and GPx, but it reduced the concentration of MDA significantly when compared with the diabetic control group ($p < 0.05$) (Table 3.4).

Table 3.4: *In Vivo* Antioxidants Analysis

Activity				**Group**		
	Control	**Diabetic Control**	**Metformin**	**100 mg of Extract**	**200 mg of Extract**	**500 mg of Extract**
SOD (unit/mg protein)	0.98±0.10	1.11±0.00	1.27±0.00	1.14±0.08	1.65±0.00*	2.66±0.00 *
Catalase (unit/mg protein)	0.37±0.067	0.07±0.00	0.47±0.06	0.49±0.06	0.82±0.00*	0.95±0.00*
GPx (U/L)	2.59±0.35	1.77±0.00	3.32±0.60	3.32±0.41	5.71±0.00 *	6.54±0.00 *
MDA (unit/mg protein)	1.50±0.15	1.33±0.00	1.66±0.42	1.86±0.14	1.24±0.00	4.18±0.00*

Values are expressed as Mean ± SEM.

Values with superscript show significant difference from the control

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.1 Discussion

The World Health Organization (WHO) describes diabetes mellitus as a chronic, progressive disease characterized by elevated levels of blood glucose which causes complications in many parts of the body and increases the overall risk of dying prematurely (WHO, 2016). This chronic non-communicable disease of the endocrine system arises from multiple etiologies in the secretion of insulin (Todkar, 2016; Omondanisi *et al.,* 2017). Diabetes mellitus describes a group of metabolic disorders characterized by a state of chronic hyperglycemia due to defects in insulin secretion, insulin action or both, which currently affects about 463 million people worldwide (Ozougwu *et al.,* 2013; IDF, 2019). Over time, elevated blood glucose, a common effect of uncontrolled diabetes mellitus, may lead to serious damage to the heart, blood vessels, eyes, kidneys and nerves (WHO, 2016)." This metabolic disorder is often described as "*a silent killer*" since it may be asymptomatic at onset. Therefore, the disease usually goes undiagnosed until major complications arise. The disease is associated with reduced life expectancy, significant morbidity as well as diminished quality of life (Abbott *et al.,* 1990; Barceló and Rajpathak, 2001; Funke and Melzig, 2006).

It has been reported that 10 % of global health expenditure is spent on diabetes (Cho *et al.,* 2018; IDF, 2019). There is profound reason to suggest that this figure might increase in the coming years giving the myriad of complications that result from diabetes mellitus. Unfortunately, four out of five people with diabetes are living in low and middle-income countries, where healthcare budgeting is not even prioritized (IDF, 2017). The lack of prioritization of healthcare in these

countries consequently creates a yawning gap in efforts aimed at possibly managing and tackling the disease scourge."

Diabetes mellitus has ceased to be a disease of affluence and has become a disease of globalization. Currently, two out of three people with diabetes are living in urban areas of the world (IDF, 2019). Therefore, it is a considered opinion that the enormous increase in prevalence of cases of diabetes mellitus may not be unrelated to factors such as increasing urbanization, high prevalence and low incidence of the disease, increasing prevalence of overweight and obesity, lack of physical activity as well as changes in socio-demographic characteristics of the population (Waugh *et al.,* 2013; Saleem *et al.,* 2017).

Many of the drugs currently used for the treatment of diabetes mellitus produce adverse effects: sulfonylureas stimulate pancreatic islet cells to secrete insulin, while metformin slows down hepatic glucose production (Moller, 2001). All these therapies have limited effectiveness, thereby necessitating the search for novel plant-based compounds that can effectively reduce blood glucose.

Diabetes mellitus is induced experimentally using different meand, one of which is STZ. Streptozotocin (STZ) 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. 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. Nitric oxide is an important messenger molecule involved in many physiological and pathological processes in the body (Busineni *et al*., 2015)**.**. Streptozotocin is widely used to induce diabetes in rodent models by inhibition of β-cell O-GlcNAcase; streptozotocin (STZ) 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. The current use of streptozotocin is mostly as an investigational drug for diabetes research due to its specific toxicity associated with pancreatic β-cells (Busineni *et al*., 2015).

Increasing evidence suggest that oxidative stress plays a role in the pathogenesis of diabetes mellitus and its complications. Hyperglycemia increases oxidative stress, which contributes to impairment of processes that fail during diabetes (insulin action and secretion). In addition, antioxidant mechanisms are diminished in diabetic patients, which may further promotes oxidative stress (Brownlee, 2001; Sanders, 2003; Rains and Jain, 2011).

The use of plant-derived compounds with antioxidant properties is of great interest to Scientists due to the overwhelming evidence implicating oxidative stress in the pathogenesis of some diseases (Bhaskaran *et al.,* 2017). Similarly, the limitations (such as a high level of toxicity and low aqueous solubility) observed following the use of synthetic antioxidants in preventing some diseases have shifted the attention of researchers to the use of naturally derived antioxidants, owing to their high safety margin, cultural acceptability, and promised effectiveness (Bandyopadhyay *et al.,* 2004; Khalil *et al.,* 2015). The aim of this study was to evaluate the antioxidant properties of methanol extract of the leaves of *Justicia carnea* in Wistar rats. The results showed that STZ-induced diabetes mellitus caused more reactive oxygen species to be generated and less antioxidants to be made. However, treatment with methanol extract of J. *carnea* leaves led to significant increases in the activities of catalase, SOD and GPx, and a marked reduction in MDA concentration of diabetic rats.

4.2 Conclusion

This study has provided evidence to support the claim that methanol extract of *J. carnea* is effective in ameliorating oxidative stress induced by STZ in diabetic Wistar rats.

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