RENAL FUNCTION IN DIABETIC WISTAR RATS TREATED WITH ETHANOL EXTRACTS OF *Cucumis sativus*

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SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF A BACHELOR OF SCIENCE DEGREE. (B.Sc. HONS)

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CERTIFICATION

This is to certify that this project work was carried out by DAFEJAIYE ANTHONIA OMOME with matriculation number, LSC1705081, under the supervision of PROFESSOR I. O. ONOAGBE and was submitted to the DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF BENIN in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.). degree in the department.

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DEDICATION

This project is dedicated to God Almighty who helped, strengthened and sustained me through the completion of this research work in good health.

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I would like to offer my special thanks to the Almighty God who has been my source of wisdom, understanding, knowledge and strength throughout this program and on his wings only have I soared.

My heartfelt gratitude goes to my parents, Mr. and Mrs. Dafejaiye who has been a constant source of support and encouragement during the challenges of school and life and whose good examples have taught me to work hard for the things that I aspire to achieve.

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ABSTRACT

Many plants have the potential of preventing and treating acute and chronic diseases. The protective effect of medicinal plant is often maintained through an increased expression of antioxidants and scavenging of free radicals. *Cucumis sativus* has a long history of use in herbal medicine. Some of its active compounds have demonstrated *in vitro* anti-tumor, anti-viral and antibacterial effects. The current study was aimed at evaluating the renal protective activity of *Cucumis sativus* against streptozotocin-induced renal toxicity. A total of twenty five Wistar rats were purchased and used for this study. The animals were grouped into five of five animals each. Group 1 served as normal control and was exposed to standard diet. Group 2 was the negative control administered streptozotocin (STZ) but not treated. Group 3 was the standard drug group (administered STZ and treated with metformin). Group 4 was the first treatment group exposed to STZ and treated with 200 mg/kg bwt of the extract. Group 5 was the second treatment group exposed to STZ and treated with 300 mg/kg bwt of the extract. At the end of the study, animals were fasted overnight and sacrificed. Blood sample was collected from the abdominal aorta of the rats, put into plain containers and centrifuged at 3000 rpm for 10 min to obtain serum. The serum was further subjected to renal function assessment. Results from this study revealed that STZ elevated the levels of urea and serum electrolytes. Elevated renal indices were significantly reduced sequel to the administration of the extract and metformin. Findings from this study suggest that the ethanol extract of *C. sativus* possesses ameliorative properties and can be used in the management of STZ-related renal damage.

CHAPTER ONE

1.0 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. (Rizza *et al*, 1981). 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 (Jayakar and Suresh, 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.(Mayadas, 2003).

Diabetogenic agents such as alloxan and streptozotocin 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 (Ghorbani, 2013). *Cucumis sativus* (Cucurbitaceae) is a fruit consumed as a vegetable or used in salads. It exhibits various pharmacological activities such as hypoglycemic and hypolipidemic activity, activity against ulcerative colitis, and wound healing activity. (Patel *et al,* 2016).

1.2 Aim and Objective

1.2.1 Aim

The study was done to investigate the renal function in diabetic wistar rats treated with ethanol extracts of *cucumis sativus*

1.2.2 Objective

To determine the effect of ethanol extract of *cucumis sativus* on urea and electrolyte indices in the kidneys

1.3 Literature Review

1.3.1 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 (Abbas and Maitra, 2005).

1.3.1.2 Types Of Diabetes Mellitus

1.3.1.3 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 (Verrotti *et al*., 2012).

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. 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).

1.3.1.4 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).

1.3.1.5 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 (e.g., 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).

1.3.1.6 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.

1.3.1.6.1 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)

1.3.1.6.2 Screening Gestational Blood Glucose

1.3.1.6.2.1 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.

1.3.1.6.2.2 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.

1.3.1.6.2.3 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).

1.3.1.6.3 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 (Tieu *et al*., 2017).

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.

1.3.1.6.4 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).

1.3.1.6.5 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).

1.3.1.6.6 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).

1.3.1.6.7 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).

1.3.1.6.8 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 (≥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).

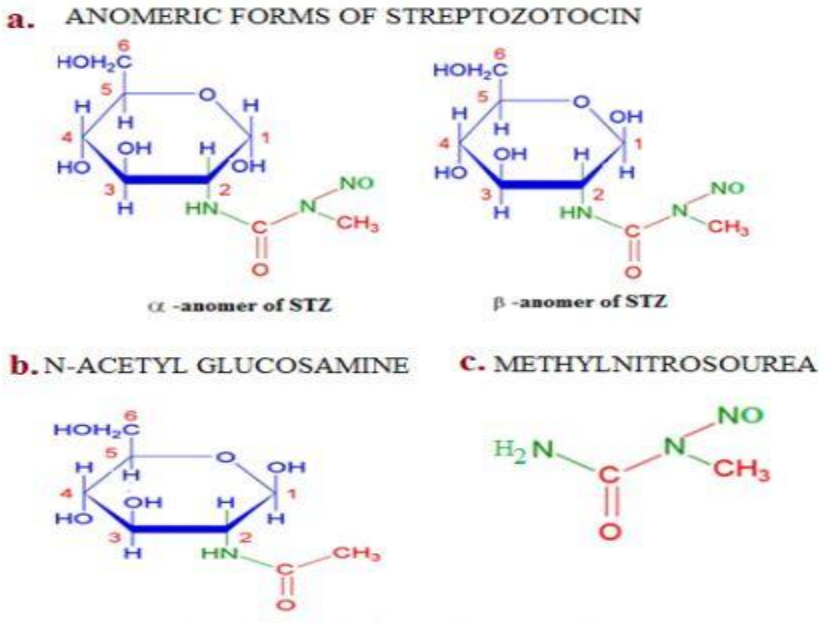


Figure 1.2

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

1.3.2.2 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 ºC for short term, but long term storage needs -20 ºC 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).

1.3.2.3 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).

1.3.3 Alloxan

Alloxan is a urea derivative used in diabetes research to induce type 1 diabetes mellitus. It has been widely used to induce diabetes in test compounds such as rabbits, dogs, rats and mice. (Iranoye *et al*, 2011). The dose required for inducing diabetes relies on the animal species and route of administration. Alloxan has been showed to be non-toxic to human's beta cells even in high doses because humans have different glucose uptake mechanisms (Tyrberg *et al.*, 2001). Alloxan induction occurs in three phases. First, there is a temporary hypoglycemic phase that last for about 30 minutes noted to be as a result of the stimulation of insulin secretion that increases the concentration of insulin the plasma. The second phase happens after one hour of alloxan administration, blood glucose levels increases and plasma insulin concentration decreases at the same time. This phase is as a result of inhibition of insulin secretion from the beta pancreatic cells caused by beta cells toxicity. In the third phase, hypoglycemia occurs again with changes that are irreversible (Tasaka *et al*, 1988).

1.3.3.1 Mechanism of Action of Alloxan

Alloxan (2,4,5,6-Tetraoxypyrimidine) reacts with glucokinase inactivating it thereby forming dialuric acid which is reoxidised back to alloxan. This reoxidation reaction forms a redox cycle and produces reactive oxygen species and superoxide radicals. These superoxide radicals produce ferric ions from ferritin; these ferric ions also undergo dismutation to yield hydrogen peroxide. Alloxan also causes deoxyribonucleic acid (DNA) fragmentation and damage in pancreatic beta cells. Cytosolic calcium ions contribute to the action of alloxan by the capability of alloxan to open voltage dependent calcium channels promoting calcium entry into the pancreatic beta cells. (Park *et al*, 1995).

1.3.4 The kidneys

The kidneys are organs shaped like kidney beans, each one about 10-15cms long, located either side of the spine, deep in the abdomen. The main job of the kidney is to cleanse the blood of toxins and transform the waste into urine. The kidneys filter the blood to get rid of waste products of metabolism, keep the electrolytes and water content of the body constant and secrete a number of essential hormones like calcitriol and renin.

Renin plays a role in maintaining blood pressure, sodium reabsorption and potassium secretion. Calcitriol a derivative of vitamins helps in maintaining normal levels of calcium and phosphorus in the blood. After food is broken down and energy is generated, waste products are sent to the blood. The kidneys are responsible for eliminating these waste products from the body and returning important metabolites like amino acids, hormones and glucose after filtration. The functional unit of the kidney is called nephron an the kidney contains up to a million of this functional unit. The nephron is made of the glomerulus and tubules. The glomerulus filters the blood while the tubules return the needed nutrients and eliminate waste.

The kidney also plays a role in salt and water balance. This balance is attributed by series of hormones. The kidney excretes excess salts from the body by series of messages sent from the brain that tells the body to drink more water, causing the kidneys to excrete urine with the excess salts.

1.3.4.1 The kidneys and Diabetes Mellitus

Diabetic nephropathy is the most chronic kidney disease caused by diabetes. Presence albumin in the urine is one of the earliest signs of diabetic nephropathy. Normally, the kidney does not excrete albumin into the urine instead it is reabsorbed back by the renal tubules. Diabetic

nephropathy is characterized by the accumulation of the extracellular matrix in the membrane of the glomerulus and tubules causing glomerular fibrosis. Diabetes is characterized by hyperglycemia; kidney structures are vulnerable to hyperglycemia causing organ damage. (Sanchez and Sharma,2009). Some studies have showed that inflammatory markers like cytokines, growth factors, chemokines, T lymphocytes and macrophages play a role diabetes mellitus as a result, diabetic nephropathy. (Sanchez and Sharma, 2009). Macrophages are recognized as the main inflammatory cell involved in kidney damage, their accumulation cause severe diabetic nephropathy in experimental models. Macrophages promote inflammatory response by increased production of reactive oxygen species (ROS). (Lopez-Parra *et al*, 2012). T lymphocytes also contribute to early kidney damage in diabetic nephropathy. Studies showed an increase in CD4 and CD8 lymphocytes in diabetic animal model and these changes were observed in the glomeruli. (Moon et al, 2012). In type 2 diabetes mellitus, T lymphocytes are over expressed by hyperglycemia that activates nuclear factor kappa B (κB). (Lei et al, 2014). Monocyte chemo attractant protein (MCP-1) a type of chemokine promotes atherosclerosis in vascular walls of epithelial cells of the tubules. Studies have also showed that patients with urinary albumin excretion presented higher circulating levels of MCP-1. (Takebayashi et al, 2006).

1.3.5 *Cucumis sativus*

Cucumber (*Cucumis sativus*) is cultivated around the world because it thrives in both temperate and tropical region resulting in its availability throughout the year. Cucumber is the fourth most

cultivated vegetable crop in the world and China is the world's largest producer of it. *Cucumis sativus* belongs to the gourd family of Cucurbitaceous, which also includes pumpkins, melon and water melon. It is cylindrical in shape and can be as large as 60 cm long and 10cm in diameter. It has dark green skin, crispy moisture flesh and small edible seeds centered in the fruit. *Cucumis sativus* is best eaten when unripe because of its crunchy and unique taste. When matured, the

skin of the fruit becomes tougher, turns yellow white accompanied with a bitter and sour taste. Studies have shown that cucumbers contain lignans, vitamin K, cucurbitacins, flavonoids, antioxidants and B vitamins among other trace elements and minerals. These substances only constitute 5% as cucumber is made up of 95% water. (Maheshwari *et al*, 2014).

Figure 1.1Cucumber fruit and leaves

1.3.5.1 Scientific classification of *Cucumis sativus*

Kingdom: Plantae

Order: Cucurbitales

Family: Cucurbitaceae

Genus: Cucumis

Specie: *Cucumis sativus*

1.3.5.2 Therapeutic Use of Cucumis sativus

Although some of these claims are still undergoing investigation, cucumber has shown to contain phytochemicals that possess anticancer, antioxidant and anti-inflammatory properties. (Kumar et al, 2010). It has also shown to improve plasma lipid and function as an analgesic. (Abu-Reidah *et al*, 2012). The high content of water in cucumbers makes it a good source of hydration. Consumption of cucumbers provides the cells hydration and important nutrients for suitable cellular functioning and maintenance of membrane integrity.(Murad and Nyo, 2016). The high dietary fiber content and low calorie value due to low carbohydrate, protein and fat content present in cucumber makes it a good choice in weight loss regimen. Cucumbers also enhance digestion because of its high fiber content. Proper digestion has been associated with easier weight loss. (Maheshwari *et al*, 2014). The cucurbitans present in cucumber present in cucumber trigger the release of insulin and regulating the metabolism of glucagon in hepatic glycogen.(Kaushik *et al*, 2015). Cucumbers are good sources of dietary fiber, particularly in their skins. Dietary fibers are known to significantly reduce the absorption of dietary cholesterol thus positively modifying the blood lipid profile with an attendant reduction in cholesterol buildup in the arteries. More so, cucumbers provide electrolyte like potassium that may contribute to preventing high blood pressure. (Yang *et al*, 2011).

CHAPTER TWO

2.0 MATERIALS AND METHODS

2.1 Materials and Apparatus

- *Cucumis sativus*
- 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

2.1.2 Chemicals And Reagents

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

- Ethyl-Acetate
- Methanol
- Alloxan
- 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

2.1.3 Collection of Materials

The fruit Cucumis sativus was obtained in a major market in Benin City, Edo state and was identified by Dr Niyi Akinnibosun of the Department of plant biology and biotechnology, University of Benin.

2.2 Methodology

2.2.1 Preparation Of Plant Extract

The fruit Cucumis sativus was collected and sliced in to thin pieces with a knife. It was then dried under low light and pulverized into a coarse powder of about 1mm in diameter. The powder was extracted by maceration in 80% ethanol for 72hours, however, with intermittent shaking at 2hours interval. The extract was then filtered using Whatman's filter paper and afterwards dried.

This powder extract was then subjected to further extraction using methanol under 72hrs. Afterwards, it was filtered using the Whatman's filter paper and the filtrate, frizzle dried. In a refrigerator at 4°C, the concentrated extract was stored until the time of use.

The formula below was used to calculate percentage yield;

$$\text{Percentage yield} = \frac{\text{Weight of extracted material}}{\text{Weight of plant material used}} \times \frac{100}{1}$$

2.2.2 Experimental Animals

Wistar rats, an outbred strain of albino rats belonging to the species "Rattus norvegicus" was the experimental animal used for this project. The animals were bred and housed in the laboratory animal House in the Department of Biochemistry, University of Benin, Benin city. They were

kept in clean, simple cages with proper ventilation and a 12hour light-dark cycle. In these cages, they were fed with standard rat pellets from Vital Feeds Nigeria which was always provided for them alongside water. The ethical guidelines for assessment of animal's behavior were strictly followed and this research was conducted in accordance with the principles for laboratory animal use and care.

2.2.3 Experimental Design

Twenty five rats were used for this experiment and they were divided randomly into five groups with each group containing five rats each. The first group termed “normal control” were not induced with streptozotocin (STZ), they only received feed and water. The second group termed “negative control” was induced with streptozotocin and left untreated. The third group termed “metformin control” was induced with streptozotocin and afterwards treated with metformin an antidiabetic drug through oral administration. The fourth group termed “Test control 1” was induced with streptozotocin and became diabetic; they were later treated with 200mg/kg of ethanol extract of the fruit Cucumis sativus via oral administration. The fifth group termed “Test control 2” were induced with streptozotocin and later administered 300mg/kg of ethanol extract of the fruit Cucumis sativus orally.

A table showing the detail is seen below;

	Normal control(Group 1)	Negative control(Group 2)	Metformin control(Group 3)	Test control (Group 4)	Test control (Group 5)
Feed	Administered	Administered	Administered	Administered	Administered
Water	Administered	Administered	Administered	Administered	Administered
Streptozotocin	_	_	Administered	Administered	Administered
Metformin	_	_	Administered	_	_
Cucumis sativus fruit extract	_	_	_	Administered	Administered

2.2.4 Induction of Diabetes Mellitus

Diabetes mellitus was induced by injecting 100mg of freshly prepared streptozotocin (STZ) which was diluted in 10 ml of normal saline, equivalent to 100mg/kg in a single dose. Before the dose was administered intraperitoneally, baseline testing was carried out. It was then administered according to body weight within a few minutes of the STZ preparation.

After a few days, decrease in appetite for food and water, loss of weight and decrease in activity of the wistar rats was noticed. To examine the diabetic status of the rats, their blood was collected by cutting a tail end incision with sharp scissors and a reagent strip. Using a potable glucometer, the fasting blood glucose levels were measured. This was done three days post induction as the results gotten were >185mg/dl.

2.2.5 Animal Sacrifice

The diabetic wistar rats were anaesthetized using chloroform before sacrifice. Upon loss of sensation, they were dissected carefully so as not to rupture any organ. Their organs were removed and transferred into a sample which was iced immediately for storage. The organ specimens were then fixed in a neutral formal-saline fixative solution so as to preserve the tissues in a life-like state. It was taken in this state to the University of Benin Teaching Hospital histological analysis.

Blood samples were then taken from the animals via cardiac puncture into EDTA-free bottles for biochemical assay. Afterward, the blood samples were centrifuged at 3000 (rpm) revolutions for 10 min to obtain plasma which was used for liver function tests.

2.2.6 Estimation of Urea Levels

Urea is a waste product from the breakdown of proteins. It is usually passed out in the urine. A high blood level of urea indicates that the kidney may not be functioning properly. Urea is often used as a measurement of kidney function

Principleμ

This assay was carried out using Randox diagnostic kit.

Urea in the plasma is hydrolyzed in ammonia in the presence of urease. The ammonia is then measured photo metrically Berthelot's reaction.

Urea $+H_20$ $\xrightarrow{\text{urease}}$ $2NH_3 + CO_2$

NH_3 +hypochlorite + phenol $\longrightarrow$ indolephenol (blue compound)

.**Reagent composition**

Reagent 1 EDTA: 116mmol/L

Reagent 1a Urease: 1g/L

Reagent 1b Sodium nitroprusside: 6mmol/L

Reagent 2 phenol (diluted): 120mmol/L

Reagent 3 sodium hydrochloride (diluted): 27mmol/L, sodium hydroxide (0.14N)

Calibration: standard

Procedure

The procedure for urea estimation is as follows

10µL of sample was added to one test tube, 10µL of standard was added to another test tube while 10µL of distilled water was added to another test tube. 100µL of reagent one (1) was then added to all test tubes mixed and incubated for 10 minutes at 37°C. 2.5µL of reagent two (2) and reagent three (3) was added to all test tubes, mixed and incubated for 10 minutes at 37°C. Absorbance was read at 546nm of the sample (A_{sample}) and standard ($A_{standard}$) against the blank

Calculation

$$\text{Urea concentration (mmol/L)} = \frac{A_{sample} \times \text{standard concentration}}{A_{sample}}$$

2.2.7 Determination of Plasma Chloride Concentration

Principle

The method consists of the addition of chloride ion in a solution of mercuric thiocyanate and ferric nitrate. The chloride ion upsets an equilibrium established between the latter two results, thereby allowing the formation of a brown ferric thiocyanate complex which is comparatively proportional to the amount of chloride added.

Reagent Composition

Reagent blank: Ferric nitrate ($Fe(NO_3)_3.9H_20$) and concentrated nitric acid (HNO_3), color reagent: Ferric nitrate ($Fe(NO_3)_3.9H_20$) and concentrated nitric acid (HNO_3), saturated mercuric thiocyanate ($Hg(SCN)_2$) AND 6% mercuric nitrate ($Hg(NO_3).H_2O$).

Assay procedure

Plasma (0.5ml) was mixed with 15ml of color reagent, while the blank contained 0.5ml of reagent blank and 15ml of color reagent. The contents of the tubes were thoroughly mixed.

After 10 minutes of incubation at 25°C, the absorbance was read at 480nm against blank. The concentration of chloride was calculated as shown below

$$\text{Chloride concentration} = \frac{\text{Absorbance of sample}}{\text{Absorbance of calibrator}} \times \text{concentration of calibrator (mEq/L)}$$

2.2.8 Determination of Plasma Potassium Ion Concentration

Principle

Under alkaline conditions, sodium tetraphenylborate reacts with potassium ion in a sample to form the potassium tetraphenylborate which is white and small particles with low solubility.

Potassium tetraphenylborate particles are in stable suspension state in the solution. The turbidity is proportional to the potassium ion concentration in the sample.

Assay Procedure

Plasma (50uḷ) was mixed with 0.2ml color reagent, while the blank contained 50uḷ of reagent blank and 0.2ml of color reagent. The contents of the tube were thoroughly mixed.

After 5 minutes of incubation at 25°C, the absorbance was read at 450nm against blank. The concentration of potassium ion was calculated as shown below

$$\text{Potassium ion conc.} = \frac{Absorbance\ of\ sample}{Absorbance\ of\ standard} \text{ x conc. Of standard (mEq/L)}$$

2.2.9 Determination Of Plasma Sodium Ion Concentration

Principle

Sodium is precipitated as the triple salt. Sodium magnesium uracil acetate with the excess uranium then being treated with Ferro cyanide, producing a chromaphore whose absorbance varies inversely s the concentration of sodium I the test specimen.

Reagent Composition

Filtrate reagent: urancyl acetate (2.1mM) and magnesium acetate (20mM) in ethyl alcohol

Acid reagent: a dilute acetic acid

Sodium color reagent: potassium Ferro cyanide, non-reactive stabilizers and fillers

Sodium standard: sodium chloride solution (130mEq/L)

Assay Procedure

To each of the labeled test tubes, sample, standard, blank and 1.0mL of filtrate reagent was dispensed. Then, 50uḷ of plasma and standard were added to their respective tubes, while distilled water was added to the blank. The tubes were mixed and vigorously shaken for 3 minutes and then centrifuged at 1500 g for 10 minutes. Subsequently, labeled test tubes corresponding to the above filtrate tubes were arranged in a rack. Then, 1.0mL of acid reagent was added to all the tubes after which supernatant was added to the respective tubes and mixed thoroughly. Exactly 50uḷ of color reagent was added to the tubes and mixed and the absorbance was read at 550nm.

$$\text{Sodium ion conc.} = \frac{Absorbance\ of\ blank - Absorbance\ of\ sample}{Absorbance\ of\ blank - A\ bsorbance\ of\ standard}\ \text{x conc. of standar(mEq/L)}$$

2.2.10 Determination Of Plasma Bicarbonate Concentration

Principle

The bicarbonate reagent utilizes the enzymatic method developed by Forrester *et al*. in this procedure bicarbonate (HCO_3^-) and phosphoenolpyruvate (PEP) are converted to oxaloacetate and phosphate in the reaction catalyzed by phosphoenolpyruvate carboxylase. Malate dehydrogenase catalyzes the reduction of oxaloacetate to malate with the concomitant oxidation of nicotiamide adenine dinucleotide (NADH). This oxidation of NADH results in a decrease in absorbance of the reaction mixture measured biochemically at 380/410 nm proportional to the bicarbonate content of the sample.

$$PEP + HCO_3^- \longrightarrow \text{oxaloacetate} + H_2PO_4$$

$$\text{Oxaloacetate} + NADH + H^+ \longrightarrow \text{malate} + NAD^+$$

Assay Procedure

To each of the test tubes labeled test, standard and blank, 1.0mL of carbon dioxide reagent was added. All tubes were incubated for 3 min at 37°C. Then, 50uL of sample and standard were added to tubes labeled test and standard respectively, while distilled water was added was added to the blank. The solution was mixed and allowed to stand at room temperature for 5 minutes after which the absorbance was read at 340nm against the reagent blank. The concentration of CO_2 was calculated as follows;

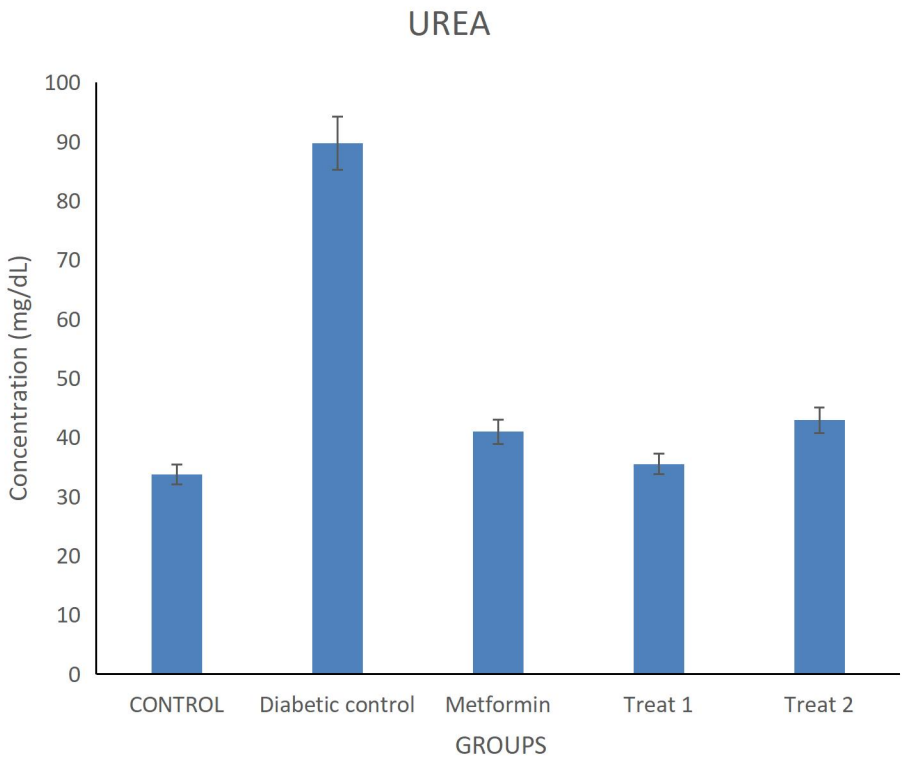
$$\text{Conc. of } CO_2 \text{ (mmol/L)} = \frac{Absorbance\ of\ blank - Absorbance\ of\ sample}{Absorbance\ of\ blank - Absorbance\ of\ standard} \text{ x conc. of standard m(Eq/L)}$$

CHAPTER THREE

3.0 RESULTS

3.1 Effect of Ethanol Extract of *Cucumis sativus* on the Concentration of Serum Urea in Diabetic Male Rats.

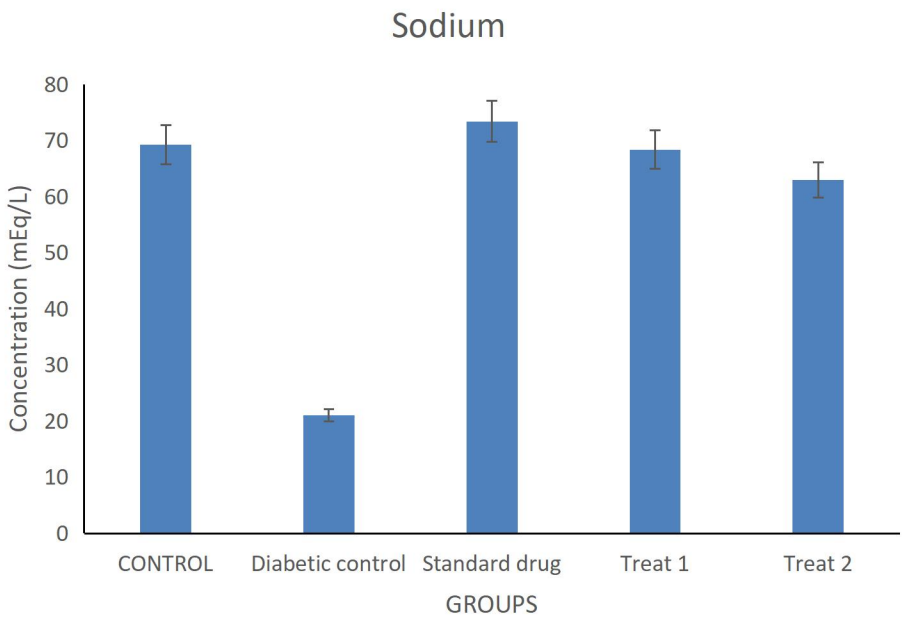
Streptozotocin-induced diabetes significantly caused a marked increase in serum urea and creatinine when compared with the control group ($p < 0.05$). Treatment with the extract and standard drug resulted in a significant reduction in urea and creatinine levels when compared with the diabetic control rats respectively ($p < 0.05$). No significant change was observed in urea in the extract-treated groups when compared to the control.



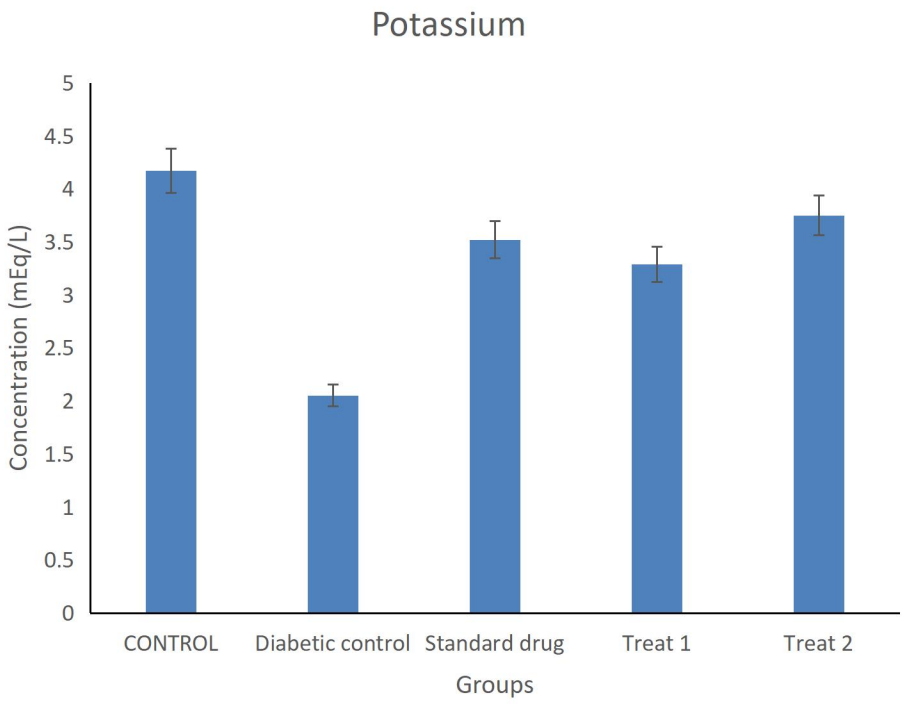
Key: Treatment 1 = 200 mg/Kg *Cucumis sativus*; Treatment 2 = 300 mg/Kg *Cucumis sativus*.

3.2 Effect of Ethanol Extract of *Cucumis sativus* on Electrolyte Indices of Diabetic-Treated Male Rats.

Streptozotocin-induced diabetes significantly caused a marked alteration in serum sodium and potassium when compared with the control group ($p < 0.05$). Graded dose treatment with the extract and standard drug resulted in a significant reduction in sodium and potassium levels when compared with the negative group respectively ($p < 0.05$). There was no statistically significant increase or decrease in chloride levels of the extract and silymarin-treated groups when compared with the positive and negative control groups ($p > 0.05$). There was a significant decrease in the levels of bicarbonate in the diabetic control treated group and the groups treated with extract when compared to the control group ($p < 0.05$). No significant increase or decrease was observed in sodium, potassium, and chloride in the extract and standard drug-treated groups when compared to the control ($p > 0.05$). There was a significant decrease in bicarbonate levels in the extract and standard drug-treated groups when compared to the control ($p < 0.05$).

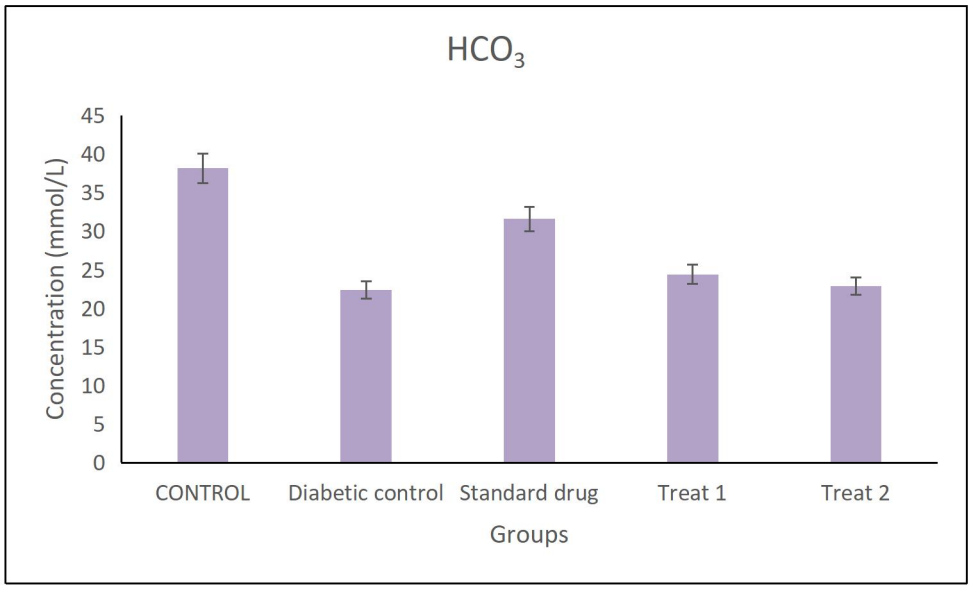


Key: Treatment 1 = 200 mg/Kg *Cucumis sativus*; Treatment 2 = 300 mg/Kg *Cucumis sativus*;



Key: Treatment 1 = 200 mg/Kg *Cucumis sativus*; Treatment 2 = 300 mg/Kg *Cucumis sativus*;

Key: Treatment 1 = 200 mg/Kg *Cucumis sativus*; Treatment 2 = 300 mg/Kg *Cucumis sativus*;



Key: Treatment 1 = 200 mg/Kg *Cucumis sativus*; Treatment 2 = 300 mg/Kg *Cucumis sativus*;

3.3 Body Weights and Blood Glucose Levels

Group	Weight Change (g)	%Weight Change	FBG (mg/dL)	Glycemic Change (mg/dL)	% Glycemic Change
Normal Control	-		-	-	-
Diabetic Control	-		> 800	-	-
Metformin	20.35	12.16	> 800	399	49.88
Treatment 1	12.26	7.87	> 800	421	52.63
Treatment 2	29.08	17.02	364	227	62.36

Data are weight and FBG parameters and are expressed as mean ± SEM (n = 5).

Group	Weight of Kidney (g)	Organ/Body Weight Ratio x 10^{-3}
Normal Control	0.73 ± 0.05	3.95 ± 0.41
Diabetic Control	0.44 ± 0.02	2.71 ± 0.17
Metformin	0.60 ± 0.30	4.08 ± 0.22
Treatment 1	0.64 ± 0. 24	3.81 ± 0.18
Treatment 2	0.62 ± 0.14	4.38 ± 0.16

Induction of diabetes mellitus using STZ significantly increased the blood glucose concentrations of the rats, but it reduced the weight of rat kidney significantly ($p < 0.05$). However, treatment of the diabetic rats with the extract markedly reduced the FBG of rats, while increasing kidney weight as well as organ/body weight ratio ($p < 0.05$).

CHAPTER FOUR

4.0 DISCUSSION AND CONCLUSION

4.1 DISCUSSION

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. The current use of streptozotocin is mostly as an investigational drug for diabetes research due to its specific toxicity associated with pancreatic β-cells.

The renal system plays a primary role in the regulation of electrolyte/fluid balance, the pH buffer system and in the elimination of waste products (Dixon, 1991). Chemical and drug-induced nephrotoxicity is a leading cause of acute kidney injury which may be due to altered intra-glomerular hemodynamic, rhabdomyolysis, inflammation, microangiopathy and tubular cell

toxicity and are been recognized as main causes of mortality and morbidity (Shahbazi *et al.,* 2012). The kidney is highly susceptible to damage by toxicants because of the high volume of blood flowing through it and the filtration of large amounts of toxins which can be concentrated in the kidney tubules (Begum *et al.,* 2011).

Findings from this result showed that intraperitoneal administration of STZ significantly elevated blood glucose and further led to an increase in the blood urea concentration of diabetic rats. Furthermore, the concentrations of serum electrolyte concentration were observed to be grossly influenced by the administration of STZ. The increases in these parameters were significantly ($p < 0.05$) ameliorated by concurrent treatment of animals with the extracts and standard drug which has protective effect against damage caused by STZ and this resulted in nearly normalcy of these parameters. This result agrees with that reported by (Kadir *et al.,* 2011). High values of blood urea and serum creatinine indicate renal damage (Khalid, 2012).

Creatinine is a nitrogenous product of metabolism in the blood, distributed throughout the total body water and are normally removed from blood by the kidney. As a measure of renal function status, increase in urea and creatinine levels could be attributed to renal damage, since kidney function with decreased blood urea and creatinine (Kingsley, 2018), these parameters are reliable kidney function markers (Gowda *et al.,* 2010). The function of the kidneys is to regulate endocrine processes such as RBC synthesis, vitamin D secretion and blood pressure maintenance (Dheer and Bhatnagar, 2010). But when the kidneys functions are affected because of complications of STZ treatment, impairment of these functions sets in. The extract was able to exert its ameliorative effect on the depreciated state of the kidney.

4.2 CONCLUSION

It can be concluded that the ethanol extract of *Cucumis sativus* possesses an ameliorative potential against STZ induced renal damage.

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APPENDICES

APPENDIX 1 (UREA)

GROUPS	UREA (mg/dl)
CONTROL	33.78 ± 6.59
Diabetic control	89.72 ± 8.55
Metformin	40.98 ± 5.26
Treatment1	35.55 ± 4.90
Treatment 2	42.92 ± 7.63

Key: Treat 1 = 200 mg/Kg *Cucumis sativus*; Treat 2 = 300 mg/Kg *Cucumis sativus*. Values are represented as mean ± SEM. Values in the same column with differ significantly ($p < 0.05$).

APPENDIX 2 (ELECTROLYTES)

GROUPS	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)	HCO_3^- (mmol/L)
CONTROL	69.29 ± 7.49	4.17 ± 0.69	51.20 ±2.54	38.15 ± 2.61
Diabetic control	21.02 ± 2.25	2.05 ± 0.08	90.52 ± 4.37	22.38 ± 1.93
Standard drug	73.41 ± 12.38	3.52 ± 0.22	46.62 ± 1.85	31.60 ± 5.37
Treatment 1	68.41 ± 4.12	3.29 ± 0.36	61.51 ± 6.28	24.41 ± 9.09
Treatment 2	63.03 ± 4.33	3.75 ± 0.29	55.07 ± 5.87	22.90 ± 2.93

Key: Treatment 1 = 200 mg/Kg *Cucumis sativus*; Treatment 2 = 300 mg/Kg *Cucumis sativus*; Na^+ = sodium; K^+ = potassium; Cl^- = chloride; HCO_3 = bicarbonate. Values are represented as mean ± SEM. Values in the same column differ significantly ($p < 0.05$).