ANTIDIABETIC EFFECT OF *Justicia carnea* IN STREPTOZOTOCIN INDUCED DIABETES IN WISTAR RATS

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CERTIFICATION

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DEDICATION

This work is dedicated to God Almighty who have helped me to come this far and to my beloved parents Mr. and Mrs. Philip Ikem for their unconditional love and support in my life.

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I want to express my profound gratitude to God Almighty for His Divine love and sustenance. My sincere gratitude goes to my loving Parents Mr. Ikem Philip and Mrs. Ikem Philomena, if not of you my educational pursuit would have been a mere dream. My sincere appreciation also goes to my loving sister, Miss Rita Ikem for her unconditional support and care, and Mrs. Deborah Okolubo of blessed memory for her encouragement and guidance in my academic pursuit. I also want to use this medium to appreciate my loving friend Miss Urhie Vwegba Martha for all her support in my academic pursuit. I won't also forget to appreciate my project supervisor, Dr. S.I. Ojeaburu for his fatherly love and support in the course of this project, and also Mr. Nathan Eimoga for his endless support and guidance all through my project research. I also thank all my project colleagues for their love and support all through the project research, I say may God Almighty bless you all in Jesus' name, amen.

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ABSTRACT

The aim of this study is to evaluate the anti-diabetic effect the *Justicia carnea* methanol extract on Streptozotocin induced diabetes in albino wistar rats. The analysis were carried out using standard biochemical methods. The oral acute toxicity test (LD50) of the *Justicia carnea* methanol leaf in rat extract was determined using Lorke's method, and diabetes was induced in the rats by a single intraperitoneal dose of 50 mg/kg. b.w of Streptozotocin. Six (6) experimental groups of rats (n=6) were used for the study. Three groups (group 4,5,6) of diabetic rats received oral daily doses of 100 mg/kg, 200 mg/kg and 500 mg/kg Methanol leaf extract of *Justicia carnea* respectively while metformin(5 mg/ml); a standard diabetic drug was administered to group 3. Group 2 was induced with diabetes but left untreated (diabetic control), while group 1 was used as normal control which was not induced with diabetes .The treatment lasted for 21days, and from the results of the acute toxicity study showed, the extract had an LD50 > 5000 mg/kg. From the result of the anti-diabetic study, a Significant ($p < 0.05$) increase was observed in blood glucose level of the untreated group (group 2) when compared with the normal control whereas groups treated with 100, 200 and 500mg/kg BW of *Justicia carnea* and 50mg/kg BW of metformin (group 3, 4, 5 and 6) showed a significant ($p < 0.05$) decrease in blood glucose levels when compared with the untreated group (group 2). Therefore, it can be concluded from the results that the leaf extracts of *Justicia carnea* can be used in the management of diabetes.

CHAPTER ONE

1.1 INTRODUCTION

Diabetes mellitus (DM) is one of the major health issue in low and middle income nations. Worldwide diabetes mellitus caused 4.3 million deaths in 2019 (Dunya *et al.,* 2022). According to recent data, 463 million adults worldwide have diabetes, and by 2045, that figure is projected to double, therefore, the illness is a public emergency (Dunya *et al.*, 2022).

Diabetes mellitus (DM) is a metabolic disease, involving inappropriately elevated blood glucose levels. It develops when the pancreas doesn't make enough insulin or any at all, or when the body isn't responding to the effects of insulin properly (Mclntyre *et al.,* 2019). Diabetes affects people of all ages. Most forms of diabetes are chronic (lifelong), and all forms are manageable with medications and/or lifestyle changes. There are several types of diabetes. The most common forms of diabetes include; type 2 diabetes in which the body doesn't make enough insulin and the body's cells don't respond normally to the insulin (insulin resistance), Prediabetes is a condition in which the blood glucose levels are higher than normal but not high enough to be officially diagnosed with Type 2 diabetes, we also have type 1 diabetes which is an autoimmune disease in which the immune system attacks and destroys insulin-producing cells in the pancreas (Dunyan *et al*., 2022). Gestational diabetes is a type of diabetes develops in some people during pregnancy. Symptoms of diabetes include; Increased thirst (polydipsia) and dry mouth, frequent urination, fatigue, blurred vision, unexplained weight loss, numbness or tingling in your hands or feet, slow-healing sores or cuts, frequent skin and/or vaginal yeast infections. Diabetes can lead to acute (sudden and severe) and long-term complications mainly due to extreme or prolonged high blood sugar levels. Acute diabetes complications that can be life-threatening include; Hyperosmolar hyperglycemic state (HHS) , diabetes-related ketoacidosis (DKA) and Severe low blood sugar (hypoglycemia) (Joanne and Jose 2020). The five main methods of managing diabetes include; Blood sugar monitoring, Oral diabetes medications, Insulin, diet, and exercise e.t.c.

The first time streptozotocin (STZ) was isolated from Streptomyces achromogenes was in 1960, but it wasn't until 1963 that its diabetogenic qualities were discussed. Based on prior research demonstrating that the diabetogenic effects are due to selective death of pancreatic islet -cells, this action was defined by Juno (Haghani *et al*., 2021).

The mice exhibit insulin insufficiency, hyperglycemia, polydipsia, and polyuria as a result of this action, all of which are signs of type 1 diabetes mellitus in humans. The pancreatic -cell cytotoxic effects of STZ are hazardous to a number of animal species, including the mouse, rat, and monkey, while being less deadly to the rabbit. Currently, STZ is most frequently used to make rats and mice develop diabetes.

Justicia carnea, also known as the Brazilian plume flower, Brazilian-plume, flamingo flower, or jacobinia, is a perennial blooming plant that is indigenous to the Atlantic Forest ecoregions of eastern Brazil (Onyeabo *et al* 2017). James Justice, a Scottish Gardner, was honored with the genus Justicia's name in the 18th century. In Nigeria, *Justicia carnea* bushes are cultivated as fences surrounding homesteads and are simple to grow from stem cuttings when the cut stems are pushed 1 to 2 inches into the soil. According to research, *Justicia carnea* is a source of both macronutrients and trace elements including calcium and iron. The study and comprehension of the chemical components of plants with therapeutic characteristics are now more popular. It is commonly used as a decorative plant in houses in Nigeria (Onyeabo *et al.,* 2017). It is widely used in eastern Nigeria as a decoction-based blood tonic. Numerous research conducted in Africa have revealed that several local plants are significant to the diet of the populace and are the most readily available and affordable sources of vital nutrients for the body. Species of medicinal plants include the elements required for therapeutic effects. The preservation of human health and wealth depends heavily on medicinal plants. The majority of people in the globe utilize herbal medications. According to reports by the World Health Organization (WHO), almost 21,000 plants have been used as medicines (Carneiro *et al*.,2023). Herbs have few negative side effects and are safe, effective, and culturally acceptable.

The use of herbal plants to enhance health has recently attracted more attention, and herbs are recognized as one of the first truly functional foods. Even if people are not concerned with the nutritional content of herbs, they are important as sources of dietary medicine and can improve the flavor and taste of our meals. Due to lack of knowledge on these plants' nutritional benefits and the presence of particular phytochemicals, they are underutilized (Onyeabo *et al.,* 2017). Some secondary metabolites, often known as phytochemicals, give plants their medicinal properties. Unawareness of these plants' nutritional benefits and the presence of some phytochemicals is the cause of their underutilization. Plants have medicinal potential due to the existence of various secondary metabolites, often known as phytochemicals (Cotoraci *et al*., 2021).

These phytochemicals may be used to create natural medications or act as building blocks for contemporary medicine. Such plants have chemical elements that are therapeutically active and have portions like leaves, roots, rhizomes, stems, barks, flowers, fruits, grains, or seeds that are utilized in the treatment of ailments (Akpovwehwee *et al.*, 2021).

Describe in this article is the antidiabetic effect of *Justicia carnea* leaf extract on streptozotocin induced diabetes in wistar rat in which a calculated amount of *Justicia carnea* leaf extract was administered routinely to the streptozotocin induced diabetic wistar rats, and the diabetic condition was totally controlled within few weeks.

1.2 Background of Study

According to Anigboro *et al., (2021), Justicia carnea* is a medicinal plant reported to have a variety of pharmacological effects, including the ability to increase blood content of the body. Many species of Justicia have historically been used to treat a variety of conditions, including inflammation, rheumatism, arthritis, liver illnesses, and gastrointestinal and diarrheal disorders. They also have analgesic, anti-inflammatory, anti-allergy, anti-tumor, and anti-viral properties, as well as antioxidant and hepatoprotective properties. According to the results of phytochemical examination, *Justicia carnea* leaves contain phenols, tannins, alkaloids, anthraquinones, saponins, flavonoids, and reducing sugars (Onyeabo *et al*.,2017). It has a long history of usage as an antibacterial, antioxidant, hypocholesterolemic, and anti-cancerous substance, which may be related to the bioactive components like phenols and flavonoids that are present in it. Diabetes mellitus is a metabolic disease characterized by hyperglycemia due to defects in insulin secretion and action or both. Changes in liver and kidney function indicators, lipid abnormalities, and anemia have all been linked to diabetes as key risk factors for the development of micro vascular and macro vascular problems.

Besides hyperglycemia, several other factors like hyperlipidemia and enhanced oxidative stress play a major role in diabetic pathogenesis, and these clinical conditions have a direct correlation with the medicinal importance of *Justicia carnea*, therefore leading to the aim of this research which is to examine the anti-diabetic effect of *Justicia carnea* on streptozotocin induced diabetes wistar rats.

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1.3 **Aims And Objectives Of Study**

This research work is aimed on the determination and evaluation of the anti-diabetic effect and property of *Justicia carnea* leaf extract on streptozotocin induced diabetes in wistar rat.

The objectives of the study include :

1) To determine the anti-diabetic effect of *Justicia carnia* in the treatment and management of diabetes, using wistar rat as an animal model.

2) To determine the anti-diabetic compositions and phytochemical components of *Justicia carnea.*

CHAPTER TWO

LITERATURE REVIEW

2.1 DIABETIS MELLITUS

Diabetes mellitus is derived from the Latin term mellitus, which means sweet, and the Greek word diabetes, which means to siphon or pass through. According to a historical analysis, Apollonius of Memphis coined the name "diabetes" somewhere between 250 and 300 BC (Oguntibeju 2019). The sweet character of the urine in this illness was discovered by the ancient Greek, Indian, and Egyptian civilizations, leading to the spread of the term diabetes mellitus. In 1889, Mering and Minkowski made the discovery that the pancreas plays a part in the pathophysiology of diabetes (Wayan *et al.,2021).*

At the University of Toronto, Banting, Best, and Collip isolated the hormone insulin from the pancreas of cows in 1922, paving the way for the creation of a successful diabetic treatment (Johanne and Jose., 2020). To address this expanding issue, great work has been done throughout the years, leading to several discoveries and the development of management techniques. Diabetes is regrettably still one of the most widespread chronic diseases in the nation and the world today. It continues to be the seventh most common cause of death in the world. Diabetes mellitus (DM) is a metabolic disease, involving inappropriately elevated blood glucose levels. DM has several categories, including type 1, type 2, maturity-onset diabetes of the young (MODY), gestational diabetes, neonatal diabetes, and secondary causes due to endocrinopathies, steroid use etc. The main subtypes of DM are Type 1 diabetes mellitus (T1DM) and Type 2 diabetes mellitus (T2DM), which classically result from defective insulin secretion (T1DM) and/or action (T2DM) (Dunya *et al*., 2022).

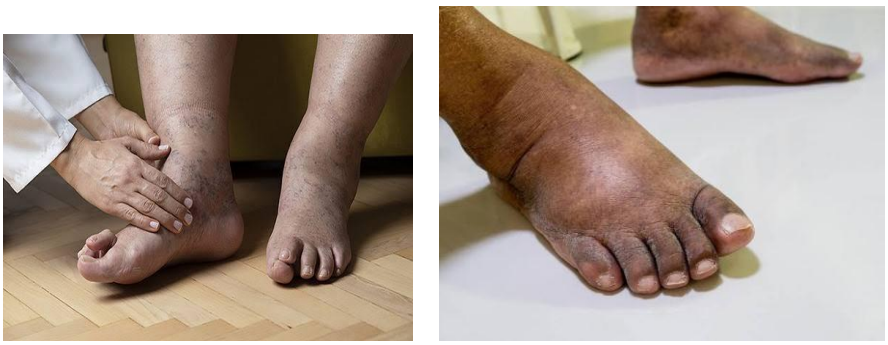

Fig 2.1 : Diagram of Patient foot with Diabetes Mellitus

Source: (Dunya *et al*., 2022).

2.1.1 Etiology

There are two primary categories of endocrine cells in the pancreatic islets of Langerhans: beta cells that produce insulin and alpha cells that secrete glucagon. Based on the glucose environment, beta and alpha cells continuously alter the amount of hormones they secrete. The glucose levels become excessively skewed when there is an imbalance between glucagon and insulin. In the case of DM, insulin is either lacking or has reduced activity (insulin resistance), which results in hyperglycemia.

T1DM is defined by the autoimmune process, which is often secondary, destroying beta cells in the pancreas. Beta cells are completely destroyed as a result, and as a result, insulin is either completely missing or very low. In T2DM, an imbalance between insulin levels and insulin sensitivity results in a functional deficit of insulin, this has a more subtly developing start (Kumar *et al*., 2020). The causes of insulin resistance are numerous, but fat and aging are the two most prominent ones. The genetic background of both types is crucial as a risk factor. Numerous loci have been identified that increase risk for DM as the human genome is studied more thoroughly. Major histocompatibility complex (MHC) and human leukocyte antigen (HLA) polymorphisms have been shown to affect the risk for T1DM (Mclntyre *et al*., 2019).

According to Habib *et al*., (2020), genetics and lifestyle play a more complicated role in T2DM. There is substantial evidence that T2DM has a greater genetic profile than T1DM. Most disease sufferers have at least one parent who has type 2 diabetes. In monozygotic twins where one twin has T2DM, there is a 90% chance that the other twin will do so as well.

Till date, 50 polymorphisms have been identified that either increase the incidence of or provide protection from T2DM. These genes produce proteins that have a role in a number of DM-related pathways, such as pancreatic development, insulin synthesis, secretion, and development, amyloid deposition in beta cells, insulin resistance, and gluconeogenesis regulatory dysfunction. Genetic loci for the transcription factor 7-like 2 gene (TCF7L2), which raises the risk for type 2 diabetes, were discovered using a genome-wide association study (GWAS). NOTCH2, JAZF1, KCNQ1, and WFS1 are other loci that may play a role in the emergence of T2DM (Habib *et al*., 2020). Due to the inherent glucogenic action of the endogenous hormones that are excessively secreted in a number of endocrinopathies, including acromegaly, Cushing syndrome, glucagonoma, hyperthyroidism, hyperaldosteronism, and somatostatininomas, these conditions have been linked to glucose intolerance and diabetes mellitus. Due to excessive pancreatic iron deposition and beta cell death, conditions like idiopathic hemochromatosis are linked to diabetes mellitus.

2.1.2 Epidemiology

90% of persons worldwide with DM have T2DM. T1DM progressively rises from infancy and reaches its peak between the ages of 4 and 6 and again between the ages of 10 and 14. A little under half (45%) of the children who arrive are under ten. About 2.3 per 1000 people under the age of 20 are affected. There are no obvious gender differences in the incidence of childhood T1DM, despite the fact that females are more likely than males to develop autoimmune disorders. Males of European ancestry who are older than 13 years old may have a higher risk of developing T1DM than females (3:2 male to female ratio) in some populations. Globally, the prevalence of T1DM has been rising. Rates are rising by 2% to 5% yearly across Europe, Australia, and the Middle East. T1DM rates increased by roughly 2% each year across the majority of age and ethnic groups in the US, and rates are greater in Hispanic kids. This pattern's precise cause is still a mystery. Some indicators, such those from the United States Military Health System data repository, however, showed a plateau between 2007 and 2012 with frequency of 1.5 per 1000 and incidence of 20.7 to 21.3 per 1000. T2DM typically develops later in life, although a rise in T2DM in younger groups has been linked to adolescent obesity (Cloete, 2021).

T2DM prevalence in the US population as a whole is at 9%, while it is closer to 25% in people over 65. According to the International Diabetes Federation, 1 in 11 persons aged 20 to 79 had diabetes worldwide in 2015. By 2040, experts predict that there will be 415 to 642 million people worldwide with diabetes, with the majority of those moving from low to middle income levels. T2DM varies by ethnic group and is 2 to 6 times more common in Blacks, Native Americans, Pima Indians, and Hispanic Americans than Whites in the United States (Cloete, 2021).

2.1.3 Pathophysiology

Since many different causes can frequently contribute to the disease, the pathophysiology of DM might be obscure. Hyperglycemia could occur in a diabetic patient. By itself, hyperglycemia can impair pancreatic beta-cell activity and cause reduced insulin production. As a result, there is vicious cycle of hyperglycemia that results in a compromised metabolic state. Blood glucose levels above 180 mg/dL are often considered hyperglycemic in this context, though because of the variety of mechanisms, there is no clear cutoff point. Patients experience osmotic diuresis due to saturation of the glucose transporters in the nephron at higher blood glucose levels (Oguntibeju, 2019). Although the effect is variable, serum glucose levels above 250 mg/dL are likely to cause symptoms of

polyuria and polydipsia. Proinflammatory cytokines and excessive fatty acids cause insulin resistance, which impairs glucose transport and speeds up fat breakdown. Due to insufficient insulin synthesis or reaction, the body responds by improperly boosting glucagon, which exacerbates hyperglycemia (Kumar *et al*., 2020).

Due to insufficient insulin synthesis or reaction, the body responds by improperly boosting glucagon, which exacerbates hyperglycemia. T2DM includes insulin resistance, but when the patient's insulin production is insufficient to make up for their insulin resistance, the condition progresses to its full extent. Nonenzymatic glycation of proteins and lipids is another effect of chronic hyperglycemia. The glycation hemoglobin (HbA1c) test can be used to determine the amount of this. Small blood vessels in the retina, kidney, and peripheral nerves become damaged as a result of glycation. Increased glucose levels speed up the process. The traditional diabetic consequences of diabetic retinopathy, nephropathy, and neuropathy as well as the avoidable outcomes of blindness, dialysis, and amputation are all caused by this damage (Kumar *et al*., 2020).

2.1.4 Types of Diabetes

Diabetes comes in a variety of forms. The most typical types include:

1) Type 2 diabetes: here the body either produces insufficient insulin or the cells don't react to it properly (insulin resistance). This form of diabetes is the most prevalent. Children can contract it, but it primarily affects adults.
2) Prediabetes: This condition is a precursor to Type 2 diabetes. Your blood glucose levels are above average but not high enough to receive a Type 2 diabetes diagnosis.
3) Diabetes type 1: This type of autoimmune illness occurs when, for an unidentified reason, the immune system assaults and kills insulin-producing cells in your pancreas. Type 1 diabetes affects up to 10% of patients with the disease. Although it can occur at any age, it is typically diagnosed in children and young people.
4) Gestational diabetes: This kind appears in certain women during pregnancy. Following pregnancy, gestational diabetes typically disappears. However, if you have gestational diabetes, you are more likely to develop Type 2 diabetes in the future.

5) Type 3c diabetes: This type of diabetes develops when your pancreas sustains injury (other than autoimmune damage), which impairs its capacity to make insulin. Damage to the pancreas that results in diabetes can be caused by pancreatitis, pancreatic cancer, cystic fibrosis, and hemochromatosis. Having a pancreatectomy also causes type 3c.
6) Latent autoimmune diabetes in adults (LADA): LADA similarly results from an autoimmune response, but it progresses much more slowly than Type 1 diabetes. The average age of those with LADA diagnoses is over 30.
7) Maturity-onset diabetes of the young (MODY): Also known as monogenic diabetes, MODY is brought on by an inherited genetic mutation that alters how your body produces and uses insulin. Right now, MODY comes in more than ten different varieties. Up to 5% of those with diabetes may be affected, and it frequently runs in families.
8) Neonatal diabetes: During the first six months of life, this unusual form of diabetes develops. It also has a monogenic diabetes subtype. The lifelong form of neonatal diabetes known as persistent neonatal diabetes mellitus affects about 50% of newborns. The illness vanishes for the other half after a few months of beginning, but it may recur later in life. Transient neonatal diabetes mellitus is the term used for this.
9) Brittle Diabetes: A type of Type 1 diabetes known as "brittle diabetes" is characterized by frequent and severe high and low blood sugar episodes. Hospitalization is frequently a result of this instability. Rarely, brittle diabetes may require a pancreas transplant to be permanently treated.

2.1.5 Assessment of Blood Glucose

People who exhibit diabetes symptoms, such as increased thirst, urine, or hunger, have their blood glucose levels checked by doctors. A patient's blood glucose levels may also be checked by a doctor if they suffer from conditions like yeast infections, ulcers on the feet, or recurrent infections that can be consequences of diabetes (Blonde *et al*., 2021). Typically, clinicians use a blood sample drawn after a person has fasted overnight to correctly assess blood glucose levels. 126 mg/dL (7.0 mmol/L) or greater fasting blood glucose levels can be used to diagnose diabetes. Blood samples collected after a meal can be used, though. Even after a meal, blood glucose levels shouldn't be particularly high. However, some elevation of blood glucose levels is normal. If a chance test results in diabetes, (not done after fasting) blood glucose level is higher than 200 mg/dL (11.1 mmol/L).

I. Haemoglobin A1C

Hemoglobin A1C, also known as glycosylated or glycolated hemoglobin, is a protein that may be measured in the blood by medical professionals. Hemoglobin A1C indicates a person's blood glucose levels over time as opposed to quick fluctuations. The oxygen-carrying, crimson component found in red blood cells is called hemoglobin. Glucose clings to hemoglobin and creates glycosylated hemoglobin when blood is subjected to high blood glucose levels over time. The blood test results for hemoglobin A1C are expressed as a percentage of hemoglobin. Hemoglobin A1C measurements can be used to identify diabetes when performed by a qualified laboratory (not by devices used at home or in a doctor's office). Diabetes is diagnosed in those with hemoglobin A1C levels of 6.5% or higher. The risk of having diabetes is increased if the level is between 5.7 and 6.4 (Blonde *et al*., 2021).

Fig 2.2: Assessment of Blood Glucose

Source: (Blonde *et al*., 2021)

II. Oral Test For Glucose Tolerance

Oral glucose tolerance tests, another type of blood test, may be used in specific circumstances, such as the screening of pregnant women for gestational diabetes or the testing of elderly individuals with signs of diabetes but normal fasting glucose levels (Podhi *et al*., 2020). However, because the test can be quite time-consuming, it is not frequently utilized for diagnosing diabetes. People who participate in this test fast, have a blood sample obtained to gauge their fasting blood glucose levels, and then drink a special solution containing a sizable

amount of glucose. The following two to three hours will see the collection of more blood samples, which will be analyzed to see if the blood glucose levels increase to excessively high levels.

III. Screening for diabetes

A standard physical checkup frequently includes a test to measure blood glucose levels. Because diabetes is so prevalent in older individuals, it is crucial to periodically check blood glucose levels in this population. People may not even be aware that they have type 2 diabetes .Even in those who have a high risk of developing type 1 diabetes (such as siblings or children of those who already have the disease), doctors do not perform routine tests to screen for the disease. However, it is crucial to do screening tests in those at risk for type 2 diabetes, such as those who;

- Are 35 years or older
- Have overweight or obesity
- Have a sedentary lifestyle
- Have a family history of diabetes
- Have prediabetes
- Have had diabetes during pregnancy or had a baby who weighed more than 9 pounds (4,000 grams) at birth (Podhi *et al*., 2020).
- Have high blood pressure
- Have a lipid disorder such as high cholesterol
- Have cardiovascular disease
- Have fatty liver disease
- Have polycystic ovary disease
- Have racial or ethnic ancestry that is associated with high risk
- Have HIV infection

Those who have these risk factors ought to have a diabetic screening at least once every three years. A risk calculator provided by the American Diabetes Association can also be used to evaluate diabetes risk. Doctors can do an oral glucose tolerance test; evaluate fasting blood glucose levels and hemoglobin A1C levels, and more. Doctors perform screening exams more frequently—at least once a year—if the test findings are on the cusp of normal and abnormal (Choudhury *et al*., 2021).

2.1.6 Common Causes of Diabetics

i. Causes Of Type 1 Diabetes

The immune system's destruction of the insulin-producing cells in the pancreas results in type 1 diabetes. By preventing the body from producing enough insulin to maintain normal function, this leads to diabetes. Because the body is attacking itself, this is referred to as an autoimmune reaction or autoimmune cause. Although there is no known cause for diabetes, the following factors may contribute:

- Viral or bacterial infection
- Chemical toxins within food
- Unidentified component causing autoimmune reaction
- Underlying genetic disposition may also be a type 1 diabetes cause.

ii. Causes of type 2 diabetes

The causes of type 2 diabetes are typically complex, meaning that multiple diabetes causes are present. A history of type 2 diabetes in the family is frequently the most discouraging aspect. The most likely cause of type 2 diabetes is this. There are numerous type 2 diabetes risk factors, and any one or more of them can raise the likelihood of getting the disease.

These consist of:

- Obesity
- Having a sedentary way of life
- advancing age
- poor diet
- Other type 2 diabetes causes such as pregnancy or illness can be type 2 diabetes risk factors

iii. Causes Of Gestational Diabetes

The root causes of gestational diabetes, commonly known as pregnancy diabetes, are yet unknown. However, a variety of risk factors raise the possibility of having this condition:

- gestational diabetes runs in families
- fat or overweight
- has polycystic ovary syndrome
- had a huge baby (more than 9 lbs)
- Ethnicity may also play a role in gestational diabetes causes; some ethnic groups are more likely to develop the condition.

iv. Additional Factors Contributing To Diabetes

Various other factors could also contribute to diabetes. The following is an example of these:

- Pancreatitis or pancreatectomy: Having a pancreatectomy and having pancreatitis are both known to raise the chance of getting diabetes (Joanne and Jose, 2020).
- PCOS: PCOS stands for polycystic ovarian syndrome. Obesity-related insulin resistance, which also raises the risk of developing type 2 diabetes and pre-diabetes, is one of the main causes of PCOS (Joanne and Jose, 2020).
- Cushing's disease: This syndrome causes an increase in cortisol hormone production, which raises blood glucose levels. Diabetes can be brought on by an excessive cortisol level.
- Glucagonoma: Due to an imbalance between the levels of glucagon and insulin synthesis, patients with glucagonoma may develop diabetes.
- A rare kind of diabetes called "steroid diabetes" develops if glucocorticoid medication is used for an extended period of time.

2.1.7 Complication of Diabetes

I. Acute diabetes complications

Acute diabetic complications include the following and pose a risk to life:

- Hyperosmolar hyperglycemic condition (HHS): People with Type 2 diabetes are primarily affected by this consequence. It occurs when your blood sugar levels are very high (above 600 mg/dL for a prolonged period), which causes severe dehydration and confusion. It needs emergency medical attention (Galicia –Garcia *et al*., 2020).
- Diabetes-related ketoacidosis (DKA): People with Type 1 diabetes (T1D) or undiagnosed T1D are most susceptible to this consequence. When your body doesn't have enough insulin, it occurs. Without insulin, your body must break down fat since it cannot use glucose as an energy source. The ketones that are finally released throughout this process make your blood acidic. Breathing difficulties, vomiting, and unconsciousness are the results of this. DKA necessitates prompt medical attention (Galicia –Garcia *et al*., 2020).

II. Severe low blood sugar (hypoglycemia): Hypoglycemia happens when your blood Sugar level drops below the range that's healthy for you. Severe hypoglycemia is very low blood sugar. It mainly affects people with diabetes who use insulin. Signs include blurred or double vision, clumsiness, disorientation and seizures. It requires treatment with emergency glucagon and/or medical intervention.

III. Hypoglycemia or extremely low blood sugar: This is a condition where your blood sugar level falls below what is considered healthy for you. An extremely low blood sugar level is severe hypoglycemia. Insulin-using diabetics are primarily impacted. Clunkiness, disorientation, double vision, and convulsions are symptoms. It necessitates either medical intervention or the administration of emergency glucagon.

IV. Long term diabetes complications

Long-term high blood glucose levels might harm the cells and organs in your body. This is primarily caused by harm to your blood vessels and nerves, which sustain the tissues in your body. The most typical sort of long-term diabetes complication is one involving the cardiovascular system (heart and blood vessels). They comprise:

- Coronary artery disease.
- Heart attack.
- Stroke.
- Atherosclerosis.
- Nerve damage (neuropathy), which can cause numbness, tingling and/or pain.
- Nephropathy, which can lead to kidney failure or the need for dialysis or transplant.
- Retinopathy, which can lead to blindness.
- Diabetes-related foot conditions.
- Skin infections.
- Amputations.
- Sexual dysfunction due to nerve and blood vessel damage, such as erectile dysfunction or vaginal dryness.
- Gastroparesis.
- Hearing loss.
- Oral health issues, such as gum (periodontal) disease.

2.1.8 Effect of Diabetes On Various Organs

- KIDNEY

The kidneys are paired organs that are primarily responsible for maintaining homeostasis and producing urine. It carries out the three basic processes of filtration, reabsorption, and secretion. Diabetes the most typical reason for renal failure. Diabetes, even when it is under control, can cause the failing of the kidneys due to chronic renal disease. The majority of diabetics do not develop chronic renal failure. When blood sugar levels are high for an extended length of time, the nephrons are destroyed and affect how well the kidneys can filter blood.

- HEART

Due to the obstruction of blood arteries by too much blood sugar, long-term diabetes infection reduces blood flow to the heart, which raises the risk of suffering a heart attack. Heart attack happens when the blood flow to one of the heart's blood arteries is suddenly cut off causing the heart muscle to suffer harm. Heart attack symptoms can include significant central chest pain and breathing difficulties, which may spread to the neck or the left arm. Some Diabetes can cause nerve damage, which makes it possible for patients to experience no heart attack symptoms.

- LIVER

The liver is one of the body's many systems that is impacted by this metabolic condition. Insulin resistance is the primary cause of hyperglycemia, which alters how lipids, carbs, and proteins are metabolized and can lead to contribute to fatty liver disease that is not caused by alcohol, which can thereafter develop into non-alcoholic cirrhosis, steatohepatitis, and lastly hepatocellular carcinomas. The fundamental process of Combined with an increase in oxidative stress, diabetes is a factor in liver damage and abnormal inflammatory response; this prompts pro-apoptotic gene production; damages hepatocytes. Liver damage is a serious complication among patients with Diabetes Mellitus. Insulin resistance, which is aggravated by oxidative stress and aberrant inflammatory signals, has become one of the main factors contributing to liver damage. Insulin resistance subsequently leads to more chronic and potentially fatal conditions such as cirrhosis and end-stage liver failure. A research conducted by Joanne and Jose (2020) showed that numerous liver abnormalities have been linked to diabetes mellitus, including aberrant glycogen deposition, non-alcoholic fatty liver disease (NAFLD), fibrosis, cirrhosis, hepatocellular carcinomas (HCCs), abnormally high hepatic enzymes, acute liver illness, and more. Furthermore, a significant buildup of fat in the liver may cause severe metabolic dysfunction and aggravate insulin resistance.

2.1.9 Treatment of Diabetes Mellitus

Before insulin was isolated in the 1920’s, the majority of patients passed away soon after their symptoms first appeared. Diabetes that is not treated results in ketoacidosis, which is an accumulation of acid and ketone bodies in the blood. As these byproducts of disordered

carbohydrate and fat metabolism continue to accumulate, they cause nausea and vomiting and eventually put the patient into a diabetic coma (Wayan *et al*., 2021).

The goal of diabetes mellitus treatment is to bring blood glucose levels down to normal ranges. Achieving this is crucial for boosting wellbeing and reducing the emergence and progression of diabetes' long-term consequences. HbA1c measurements can be used to judge how well a person is responding to their diabetic medication. HbA1c target results should be within a few points of normal (Oguntibeju, 2019).

1) Diet and exercise

All diabetes patients are placed on diets intended to help them achieve and maintain a healthy body weight, and they are frequently urged to engage in regular exercise, which improves the transport of glucose into muscle cells and dampens the increase in blood glucose that occurs after carbohydrate absorption. Patients are urged to eat a diet that is moderately low in fat and has enough protein. In actuality, roughly 30% of calories should be from fat, 20% from protein, and the remaining 60% from carbohydrates, preferably complex carbohydrates rather than simple sugars. Based on the patient's nutritional needs for growth or, if the patient is obese, for weight loss, the total calorie content should be calculated. Caloric restriction for even a short period of time in patients with type 2 diabetes who are overweight or obese can significantly reduce hyperglycemia. Additionally, losing weight—ideally in conjunction with exercise—can enhance insulin sensitivity and potentially restore normal glucose metabolism.

2) Insulin Therapy

Insulin therapy is necessary for diabetics whose bodies are unable to manufacture insulin. Regular insulin injections are required as part of traditional insulin therapy and these injections are frequently tailored to the patient's unique and changing needs. The pancreatic extracts of cattle or pigs are used to make beef or pork insulin, which is used to treat diabetic people. In contrast, the production of insulin derived from beef and pork has been abandoned in the United States in favor of human insulin. Recombinant DNA technology is the foundation of contemporary human insulin therapy (Kumar *et al*., 2020). Human insulin can be administered in two different forms: short-acting insulin, which works fast but only temporarily, and long-acting insulin, which has been biochemically altered to extend its effect for up to 24 hours. 2014 saw the release of an inhalable form of another type of insulin that has a quick onset of action, beginning to decrease blood sugar within 10 to 30 minutes of administration. The optimal regimen is one that most closely mimics the normal pattern

of insulin secretion, which is a constant low level of insulin secretion plus a pulse of secretion after each meal. This can be achieved by administration of a long-acting insulin preparation once daily plus administration of a rapid-acting insulin preparation with or just before each meal. Patients also have the option of using an insulin pump, which allows them to control variations in the rate of insulin administration. A satisfactory compromise for some patients is twice-daily administration of mixtures of intermediate-acting and short-acting insulin. Patients taking insulin also may need to vary food intake from meal to meal, according to their level of activity; as exercise frequency and intensity increase, less insulin and more food intake may be necessary (Habib *et al*., 2020).

The best regimen is one that most nearly resembles the natural rhythm of insulin secretion, which is a pulse of insulin secretion after each meal and a continual low level of secretion. This can be accomplished by giving a rapid-acting insulin preparation with or right before each meal in addition to giving a long-acting insulin preparation once daily.

Additionally, patients have the choice to use an insulin pump, which gives them the ability to regulate changes in the rate of insulin supply. The twice-daily prescription of blends of intermediate-acting and short-acting insulin is a reasonable solution for certain people. Depending on their degree of activity, patients using insulin may also need to adjust their food intake from meal to meal; as exercise frequency and intensity rise, less insulin. Research into other areas of insulin therapy include pancreas transplantation, beta cell transplantation, implantable mechanical insulin infusion systems, and the generation of beta cells from existing exocrine cells in the pancreas. Patients with type 1 diabetes have been treated by transplantation of the pancreas or of the islets of Langerhans.

However, limited quantities of pancreatic tissue are available for transplantation, prolonged immunosuppressive therapy is needed, and there is a high likelihood that the transplanted tissue will be rejected even when the patient is receiving immunosuppressive therapy (Blonde *et al*., 2021).

Pancreas transplantation, beta cell transplantation, implantable mechanical insulin infusion systems, and the creation of beta cells from exocrine cells already present in the pancreas are some of the various approaches of insulin therapy now being researched. Pancreas or islets of Langerhans transplants have been used to treat type 1 diabetes patients. However, there is a shortage of pancreatic tissue that can be used for transplantation, long-term immunosuppressive therapy is required, and even when the patient is undergoing immunosuppressive therapy and there is a high probability that the transplanted tissue would be rejected (Habib *et al*., 2020).

3) Use Of Drug

There are several classes of oral drugs used to control blood glucose levels, including sulfonylureas, biguanides and thiazolidinediones. Sulfonylureas, such as glipizide and glimepiride, are considered hypoglycemic agents because they stimulate the release of insulin from beta cells in the pancreas, thus reducing blood glucose levels. The most common side effect associated with sulfonylureas is hypoglycemia (abnormally low blood glucose levels), which occurs most often in elderly patients who have impaired liver or kidney function. Sulfonylureas, biguanides, and thiazolidinediones are only a few of the kinds of oral medications used to manage blood sugar levels. Because they encourage the release of insulin from beta cells in the pancreas, sulfonylureas like glipizide and glimepiride are regarded as hypoglycemic drugs and lower blood glucose levels (Choudhury *et al*., 2021). Hypoglycemia (abnormally low blood glucose levels), the most frequent side effect of sulfonylureas, is most frequently experienced by elderly individuals with compromised liver or kidney function. Biguanides, of which metformin is the main component, are regarded as antihyperglycemic medications because they function by reducing the liver's synthesis of glucose and by enhancing the action of insulin on muscle and adipose tissues. Metformin side effects that can be fatal include the buildup of lactic acid in blood and tissues, which frequently results in nebulous symptoms like nausea and weakness. Thiazolidinediones, like rosiglitazone and pioglitazone, work by lowering muscle and adipose cells' resistance to insulin and by enhancing the transport of glucose into these tissues. In some patients, these medications can result in severe cardiovascular events, liver damage, and edema (fluid accumulation in tissues). Additionally, oral hypoglycemic medicines only significantly lower mean blood glucose levels by 50 to 80 mg per 100 ml (2.8 to 4.4 mmol per litre), and over time, sensitivity to these medications tends to decline. There are a number of additional medications that can be used to treat diabetes very effectively. According to Wanya *et al*., 2021, Pramlintide, a synthetic hormone can be injected and is based on the human hormone amylin which lowers blood sugar levels by suppressing glucagon, which typically drives the creation of glucose in the liver, and by decreasing food absorption in the stomach.

Exenatide is an injectable antihyperglycemic medication that functions similarly to incretins, or gastrointestinal hormones, which prompt the pancreas to release insulin. Because exenatide is more resistant to breakdown by the enzyme dipeptidyl peptidase-4 (DPP-4), it lasts longer than the body's own incretins.

Sitagliptin is a medication that specifically inhibits DPP-4, raising amounts of incretins produced by the body. Although pramlintide can induce severe hypoglycemia in people with type 1 diabetes, side effects from these medications are typically modest (Galicia – Garcia *et al*., 2020).

2.2 JUSTICIA CARNEA

Justicia carnea, the Brazilian plume flower, Brazilian-plume, flamingo flower, or jacobinia, is a flowering plant in the family Acanthaceae. *Justicia carnea*, commonly called Brazilian plume. While some of the smaller kinds only reach heights of 2 feet, flower, Brazilian-plume, flamingo flower and jacobinia belongs to the largest genus (Justicia) of the family of during the winter, placed in containers and can exhibit a magenta, orange, purple, and coral/apricot. The spike produces flowers that have a tube-like form and curl outward. Acanthaceae, which consists of about 600 species of shrubs and subtropics. The plant can be brought indoors herbs, and tender perennial commonly found in the tropics variety of summertime hues, including white, pink, red, rose, others can reach heights of 6 feet and a width of 6 feet (Onyeabo *et al*., 2017).

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 . 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 justicia ideal for use as a foundation or mass planting. It may be used in various containers, or it 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 (Anigboro *et al*., 2021).

Fig. 2.3: *Justicia carnea*

Source: (Anigboro *et al*., 2021).

2.2.1 Taxonomy

Kingdom: Plantae

Class: Tracheophytes

Clade: Angiosperms

Clade: Eudicots

Clade: Asterids

Order: Lamiales

Family: Acanthaceae

Subfamily: Acanthoideae

Tribe: Justicieae

Genus: Justicia

Species: carnea

(Okocha *et al*., 2023).

2.2.2 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

Planting month : year round

Origin: native to South America

Uses: border; mass planting; container or above-ground planter; cut flowers

DESCRIPTION

Height: 3 to 6 feet

Spread: 2 to 3 feet

Plant habit: upright; spreading

Plant density: open

Growth rate: slow

Texture: coarse

FOLIAGE

Leaf arrangement: opposite/subopposite

Leaf type: simple

Leaf margin: undulate

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

FLOWER

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

Flower characteristic: flowers periodically throughout the year

FRUIT

Fruit shape: pod or pod-like

Fruit length: less than .5 inch

Fruit cover: dry or hard

Fruit color: brown

Fruit characteristic: inconspicuous and not showy

TRUNK AND BRANCHES

Trunk/bark/branches: not particularly showy; typically multi-trunked or clumping stems

Current year stem/twig color: green

Current year stem/twig thickness: very thick

CULTURE

Light requirement: plant grows in the shade

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

Drought tolerance: unknown

Soil salt tolerances: poor

Plant spacing: 24 to 36 inches

OTHER

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

2.2.3 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. *Justicia carnea* should be grown on rich, well-drained soil that receives ample moisture in a location shaded from full-day sun. The only necessary pruning during the growing season should be limited to removal of dead flower heads or occasional pinching to stimulate branching. 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 (Okocha *et al*., 2023).

.2.2.4 PEST AND DISEASES

Nematodes and spider mites. Caterpillars occasionally chew portions of the foliage, but these will not kill the plant. No pests or diseases are of major concern (Akpovwehwee *et al*., 2021).

2.2.5 Nutritional Composition of *Justicia carnea*

Justicia carnea is full of minerals and phytochemicals, which add to its therapeutic benefits. The plant contains a variety of secondary metabolites, such as flavonoids, tannins, saponins, alkaloids, phenols, and terpenoids.

Vitamins A, B1, B12, B6, B9, and E are only a few of the many vitamins and nutrients that are present in *Justicia carnea*. For problems with the skin and nails, use this topical with high levels of vitamin C. In addition to offering further advantages, it also lowers the risk of illness that is frequently linked to fruit and vegetable juices. Minerals like calcium, magnesium, zinc, and copper are also present in the plant (Ajuru *et al*., 2022).

1) phytochemical and secondary metabolite

There are numerous secondary metabolites present in *Justicia carnea* extracts, according to numerous phytochemical investigations, which support the plant's health advantages. These consist of:

- Flavonoids: These anti-inflammatory, antiviral, and antioxidant plant chemicals.
- Tannins: Tannins have astringent and anti-inflammatory properties.
- Saponins: Saponins have a number of health advantages, including raising immunity and lowering cholesterol.
- Alkaloids: Nitrogen compounds known as alkaloids have a variety of pharmacological effects that reduce pain and inflammation.
- Phenols: Phenolic substances are substances with antioxidant qualities. They aid in defending your body and brain against oxidant-related harm. Stress and pollution are a couple of the environmental toxins that contribute to this harm. Your body may also benefit from the chemicals' anti-inflammatory properties.
- Terpenoids: Terpenoids are a broad class of plant substances that have a number of health advantages, such as anti-inflammatory, antioxidant, and anticancer activities.

2) Vitamin and Minerals

The vitamin and mineral content of *Justicia carnea* is high, supporting general health. The plant's leaf extract in water contains:

- Vitamin A: Maintaining clear vision and a strong immune system require vitamin A.
- Vitamin B: These vitamins have a critical role in cellular processes, metabolism, the production of energy, and brain activity.
- Vitamin C is an antioxidant aids in the management of your immune system and aids in iron absorption.
- Vitamin E is a beneficial dietary supplement that supports healthy skin and reproductive processes by shielding them from harm. Additionally, it promotes wholesome cell growth.
- Iron is a mineral that supports blood production, which is vital in maintaining an adequate oxygen supply. It can also help transport oxygen to other body parts and aid in transporting nutrients and waste products.
- Magnesium, Zinc, and Copper are essential in various physiological processes, including immune function and energy metabolism.

2.2.6 Benefits of *Justicia carnea*

The abundance of various vitamins, minerals, and phytochemicals adds to the many health advantages of *Justicia carnea*. Benefits of including this plant in diet are listed below:

1) Boost Blood Production:

One of the benefits *Justicia carnea* provides is a boost to the production of blood. Several studies have found that *Justicia carnea* can increase red blood cell, platelet, and hemoglobin levels in individuals - making it an invaluable resource for those with anemia or other blood disorders. Iron and vitamin C are the primary nutrients in plants, which work together to support blood production. This is primarily due to the leaf's high concentration of iron and vitamin C (Orjiakor *et al*., 2019).
The increased blood production that *Justicia carnea* offers is one of its advantages. A number of studies have revealed that *Justicia carnea* can raise people's levels of hemoglobin, red blood cells, and platelets, making it a crucial supplement for those with anemia and other blood diseases.

2) Prevents Anaemia:

Since ancient times, *Justicia carnea* has been used in traditional medicine to cure a variety of ailments, including anemia. Its effectiveness as a potential anemia therapy and prevention measure has been supported by recent studies.

A study in the year 2017 by Onyeabo *et al* confirmed the potential of plant extracts for treating and preventing this widespread ailment by showing that they could cure anemic conditions, enhance red blood cell synthesis, and improve iron absorption. This shows that *Justicia carnea* may be able to assist in treating the lipid abnormalities brought on by anemia.

3) Antidiabetic Property:

Recent studies back up the usage of the plant *Justicia carnea*, also known as Ewe Eje in Yoruba or Ogwu Obara in Igbo, in traditional diabetic treatment. In a study, it was found that a methanol extract from the *Justicia carnea* not only decreased blood glucose levels in rats with alloxan-induced diabetes, but also stopped the progression of new symptoms including diabetic retinopathy. As a result, it can have hypoglycemic and anti-diabetic properties (Orjiakor *et al*.,2019).

4) Powerful Antioxidant:

Natural antioxidants in J*usticia carnea* are quite potent. This plant, which is native to Brazil and West Africa, is a rich source of phenols, ascorbic acid, carotene, lycopene and flavonoids that can offer the best defense against oxidative damage. *Justicia carnea* has grown to be crucial for those trying to maintain their health and well-being because of its outstanding spectrum of defensive characteristics (Okutu *et al*., 2022).

5) Protect The Liver:

Justicia carnea possesses hepatoprotective qualities that help shield the liver and enhance its performance, according to research. In one study, the plant's extracts showed liver-protective benefits against rats' liver damage brought on by carbon tetrachloride. There has been a lot of research on utilizing the leaf to cure hepatitis and other liver disorders (Okutu *et al*., 2022).

6) Lowers Cholesterol:

It has been shown that *Justicia carnea* extracts contain anti-hyperlipidemic characteristics, which means they can aid in lowering high cholesterol levels. Studies have shown that the plant's leaf extract dramatically increased HDL levels while lowering serum cholesterol, LDL, VLDL, and triacylglycerol concentrations (Okutu *et al*., 2022). The consumption of *Justicia carnea* may

enhance one's health by lowering the risk of conditions including atherosclerosis, heart disease and stroke.

7) Boost Immune System:

The potential of the plant *Justicia carnea* to increase red blood cell synthesis makes it popular. *Justicia carnea* boosts the formation of white blood cells, immunity and body defenses, according to University of Michigan researchers who have investigated the plant. These antibodies and immune cells defend the body from illness and infection. As a result, drinking *Justicia carnea* may boost the immune system and enhance general health (Okocha *et al*., 2023).

8) Improve Heart Health:

Justicia carnea is a medicinal plant that has been used for many years to cure a variety of illnesses. Studies indicate that the plant can benefit cardiovascular health by lowering dangerous cholesterol levels, oxidative stress, and inflammation. Due to its antioxidant and anti-hyperlipidemic qualities, recent study has suggested that it may play a part in enhancing heart health (Ani *et al*., 2020).

9) May Possess Antidepressant Properties:

Justicia carnea may have antidepressant effects. Numerous studies on different Justicia species have indicated their potential as antidepressants, while research on its effects is still in its early stages. This has led researchers to investigate the therapeutic advantages of this plant more thoroughly, especially in view of the expanding demand for more efficient and all-natural treatments for depression (Ani *et al*., 2020).

10) May improve immunity and epileptic condition

Justicia carnea has been used in traditional medicines since ancient times. Mounting evidence suggests it may potentially boost immunity and improve epileptic conditions. Studies conducted on animals have found that *Justicia carnea* has potent anti-inflammatory and antioxidant properties, which can help strengthen the immune system (Ani *et al*., 2020). Additionally, its ability to reduce seizure activity in animal models of epilepsy makes it a promising candidate for further research into the treatment of epilepsy. The plant's bioactive compounds, such as polyphenols and antioxidants, can support immune function and protect the central nervous system from damage.

2.2.7 Phytochemical components of *Justicia carnea*

Preliminary phytochemical analysis of ethanolic extract of *Justicia carnea* showed the presence of alkaloid, steroid, flavonoid, phenol, carbohydrate, saponin and quinone.

- Alkaloid: Chemical substances known as alkaloids are byproducts of plants' secondary metabolism. They all include at least one single nitrogen atom, making them compounds with an alkaline property. Most of the alkaloids are N-heterocycliccompounds (sometimes called true alkaloids).We can also find other nitrogen-containing alkaloids, such as alkaloids derived from cyclopeptides, protoalkaloids, and amino acids (protoalkaloids), polyaminealkaloids. Compounds with steroid, purine, and terpene-like structures are among the very last group of alkaloid-like chemicals. Alkaloids are substances we encounter every day, and the majority of them have an impact on our bodies functions. A few of them are well known for their medicinal effects. While some of they are crucial medical devices while others are global widely abused harmful, addictive and dangerous drugs. Most of the alkaloid compounds are created in plants because of special method of secondary product secretion
- Steroids: A steroid is an organic molecule with four fused rings organized in a certain molecular arrangement that is biologically active. The two main biological roles of steroids are as signaling molecules and as critical elements of cell membranes that affect membrane fluidity.
- Flavonoids: Flavonoids are widely spread in plants ,they play in many functions. Flavonoids provide colored flower pigmentation that draws pollinating animals to flowers. Flavonoids play a role in higher plants' UV filtration, nitrogen fixation, and flower coloring. Flavonoids may act as a chemical messenger or physiological regulator; it is also act as cell cycle inhibitor (Onyeabo *et al*., 2017). Flavonoids secreted through the root of their host plants help in the formation of a root nodule. Some flavonoids contain inhibitory activity against organisms that cause plant illness like Fusarium oxysporum. Research has shown that flavonoids may modify allergens, viruses, carcinogens, and so it may be called as biological response modifiers.

- Saponin : Saponins are secondary metabolites derived from natural sources and are widely distributed in a variety of plant species. Saponins are amphipathic glycosides that when shaken in aqueous solutions produce foam that resembles soap.

  Structure of saponins is defined by their composition of one or more hydrophilic glycoside structure is combined with alipophilic triterpene derivative.

2.3 STREPTOZOTOCIN

Streptozotocin, also known as streptozocin (INN, USP) (STZ), is an alkylating antineoplastic substance that occurs naturally. It is especially harmful to the insulin-producing beta cells of the pancreas in animals. In addition to producing an animal model for hyperglycemia, Alzheimer's, type 2 diabetes, or type 1 diabetes with numerous modest doses, it is utilized in medicine to treat some islets of Langerhans tumors and in medical research to study these conditions (Diego *et al*., 2019).

Fig 2.4: Streptozocin bottle

Source: (Diego *et al*., 2019)

2.3.1 Usage of Streptozotocin

The U.S. Food and Drug Administration (FDA) has given streptozotocin the go-ahead to treat metastatic pancreatic islet cell cancer. Since it seldom cures cancer and entails a significant risk of toxicity, its usage is often restricted to patients whose cancer cannot be surgically removed.

Streptozotocin has the ability to lessen the symptoms in these patients, particularly hypoglycemia brought on by excessive insulin release by insulinomas with a common dose is 500 mg/m2/day administered intravenously for 5 days, with a repeat administration every 4-6 weeks (Haghani *et al*., 2021). Streptozotocin has long been employed in scientific studies to cause diabetes and lupus in experimental animals because of its extreme toxicity to beta cells. Additionally, the memory loss in mice caused by streptozotocin has been used to imitate Alzheimer's disease (Kandasamy *et al*., 2021).

2.3.2 History of Streptozotocin

The antibiotic streptozotocin was first discovered in the late 1950s. Researchers at the drug manufacturer Upjohn in Kalamazoo, Michigan, which is now a division of Pfizer, found the Blue Rapids, Kansas, can be regarded as the origin of streptozotocin because the soil sample from which the microorganism was discovered was obtained. U.S. Patent 3,027,300 was issued in March 1962 following Upjohn's August 1958 filing for patent protection of the medication (Chen and Cheng 2021).

Streptozotocin was discovered to be specifically harmful to the pancreatic islets' beta cells in the middle of the 1960s. These cells normally control blood glucose levels by secreting the hormone insulin (Diego *et al*., 2019). This indicated the use of the medication as a diabetes animal model as a therapy for beta cell malignancy. The National Cancer Institute looked into the application of streptozotocin as a cancer chemotherapeutic agent in the 1960s and 1970s. Streptozotocin was approved by the FDA in July 1982 after Upjohn requested it as a treatment for pancreatic islet cell cancer in November 1976. Later, Zanosar was used to sell the medication. Recent research has shown that type-2 diabetes, which causes insulin signaling to become dysregulated, dramatically raises the risk of cognitive decline and the progression of Alzheimer's disease (AD).On the basis of this, an animal model of brain insulin resistance has been developed using the direct administration of STZ to the brain (i.e., through intracerebroventricular (ICV) infusion) to replicate in rats the pathophysiology of sporadic AD, which represents the most prevalent form of AD in people. Amyloid beta (A) protein buildup in the brain, as well as oxidative stress and cognitive decline, were brought on by STZ infusion.

On account of this, an animal model of brain insulin resistance has been created by the direct delivery of STZ to the brain (specifically, intracerebroventricular (ICV) infusion), which mimics the pathophysiology of sporadic AD, the most prevalent kind of AD in people (Chen and Cheng 2021). Amyloid beta (A) protein buildup in the brain, as well as oxidative stress and cognitive decline, were brought on by STZ infusion. Notably, there is now proof that STZ infusion in the brain increased the levels of neuroinflammation, tau hyperphosphorylation, and amyloid precursor protein (APP) (Haghani *et al*., 2021).

2.3.3 Mechanism of Action of Streptozotocin

Glucosamine and nitrosourea make up the substance streptozotocin. Although additional mechanisms may potentially be involved, it is hazardous to cells by damaging the DNA, just like other alkylating chemicals in the nitrosourea class (Chen and Cheng 2021). Diabetes is most likely caused by PARP activation, not DNA damage, which is caused by DNA damage. Streptozotocin is similar enough to glucose to be taken up by the glucose transport protein GLUT2, but it is not recognized by the other glucose transporters. This explains why beta cells, which have comparatively high amounts of GLUT2, are particularly toxic to it (Alexandre-Silva *et al*., 2023).

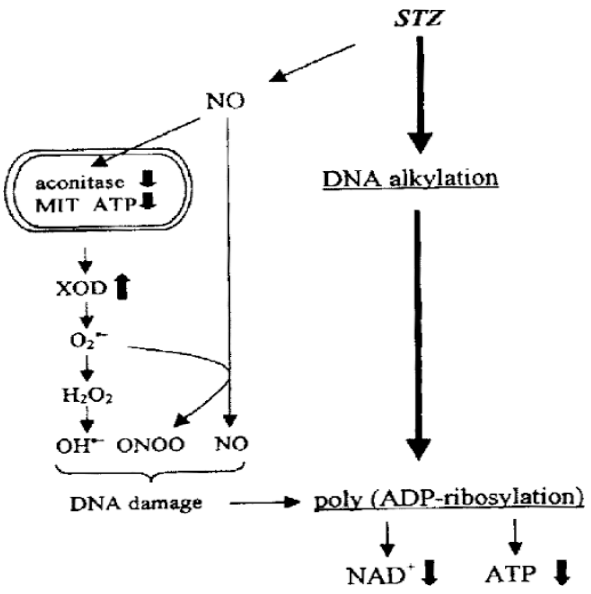

Fig. 2.5: Mechanism of Action of Streptozotocin

Source: (Alexandre-Silva *et al*., 2023).

2.3.4 Chemical Properties of Streptozotocin

A. It is a cytotoxic methyl nitrosourea moiety (N-methyl-N-nitrosourea) attached to the glucose (2-deoxyglucose) molecule.
B. It is a glucosamine derivative.
C. It is a toxic beta cell glucose analogue.
D. It is a hydrophilic compound.
E. It is an alkylating agent.
F. STZ is a toxic glucose (Glu) and N-acetyl glucosamine (GlcNAc) analogue that is accumulated preferentially in pancreatic β-cells via GLUT 2 transporter uptake.
G. It is relatively stable at pH 7.4 and 37°C at least for up to I hr.
H. It has a biological half life of 5–15 minutes.
I. When reconstituted into a solution, it can be stored at room temperature or refrigerator but must be used within 12 hrs if stored at room temperature and protected from sunlight.

2.3.5 Elucidation of The Diabetogenic Action of STZ

Rats, mice, monkeys, hamsters, rabbits, and guinea pigs all develop diabetes when given STZ. The absorption of STZ by cells plays a role in its harmful effects. STZ, on the other hand, is a hydrophilic chemical due to hexose substitution, which inhibits its uptake by cells, whereas nitrosourea compounds are often lipophilic, making their uptake by cells very rapid. The glucose moiety in STZ's chemical structure, which allows it to enter the beta cell via the low affinity glucose 2 transporter in the plasma membrane, is related to the selective pancreatic beta cell toxicity and diabetic condition that result from STZ induction because the pancreatic -cells are more active than other cells in taking up glucose and therefore more sensitive than other cells to STZ challenge (Ya-chun *et al*., 2021). This claim is supported by the finding that insulin-producing cells that do not express this glucose transporter are resistant to STZ toxicity and only become susceptible to its toxicity following GLUT 2 transporter protein expression in the plasma membrane. The hepatocytes and renal tubular cells, two additional types that express this GLUT 2 transporter, are similarly vulnerable to STZ (Baig *et al*., 2020).

This explains why STZ-induced renal and hepatic damage occurs often in experimental animals. Additionally, after STZ challenge, non-beta cells such cells and the extra-pancreatic parenchyma remain unharmed, demonstrating the beta cell selective features of STZ.

Additionally, STZ damages cardiac and adipose tissue and heightens oxidative stress, inflammation, and endothelial dysfunction (Ya-chun *et al*., 2021). Furthermore, liver, kidney, gut, and pancreas concentrations of the medication or its metabolites are consistently higher than those in plasma. When used to treat human islet-cell carcinomas and malignant carcinoid tumors, STZ has no effect on the pancreatic beta cells. Due to the very low level of constitutive GLUT 2 transporter expression in human beta cells, the human beta cells are resistant to STZ (Sundaram *et al*., 2020).

2.3.6 Biochemical Basis of The Cytotoxicity of STZ That Results In Cell Death (Apoptosis/Necrosis)

N-acetyl glucosamine (GlcNAc) and glucose (Glu) are structural analogues of STZ. Through the GLUT 2 transporter, STZ enters pancreatic beta-cells where, as previously indicated, the nitrosourea moiety induces DNA fragmentation, leading to beta-cell death. There are three main mechanisms that lead to cell death: (i) DNA methylation, which produces the carbonium ion (CH3+), which activates the nuclear enzyme poly ADP-ribose synthetase as a component of the cell repair mechanism and subsequently causes NAD+ depletion (ii) Nitric oxide production (iii) Hydrogen peroxide's role in the formation of free radicals (Sundaram *et al*., 2020).

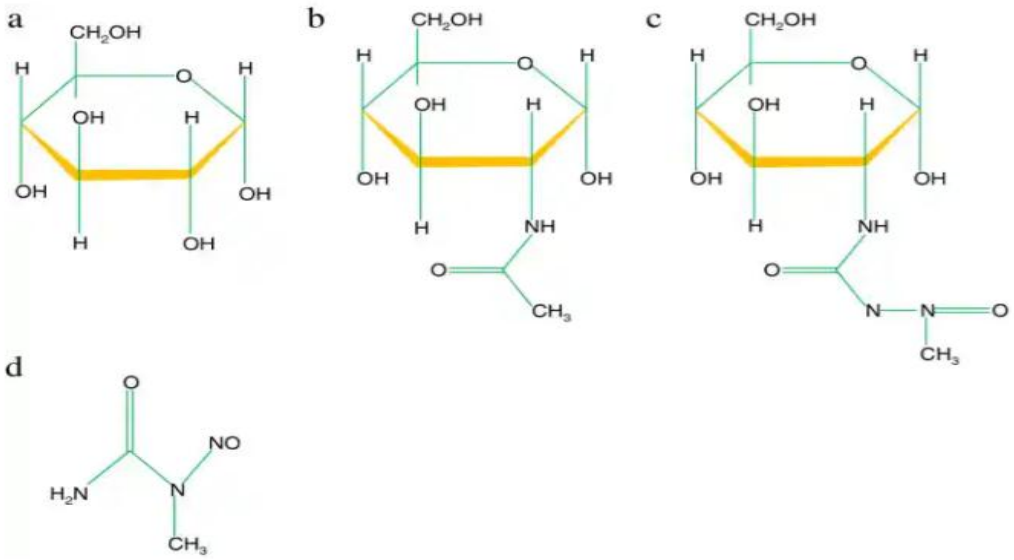

Fig. 2.6: Structures of (a) glucose (b) N-acetyl glucosamine (c) Strptozotocin (d) methylnitrosourea.

I. DNA methylation

One explanation for the cell death brought on by STZ induction is the DNA methylating activity of the methylnitrosourea moiety of STZ, particularly at the O6 position of guanine, which results in DNA damage and necrosis of the pancreatic beta cells through the depletion of cellular energy stores. Because DNA repair mechanisms are overstimulated, the subsequent activation of polyADP-ribose polymerase (PARP), in an effort to fix the damaged DNA, depletes the cellular NAD+ and ATP stores. Although STZ also methylates proteins, the majority of beta cell mortality results from DNA methylation; nonetheless, STZ's toxicity to beta pancreatic cells may also be influenced by its methylation of proteins (Chen and cheng, 2021). Additionally, nicotinamide and other inhibitors of this poly ADP ribose polymerase prevent STZ from methylating DNA. For instance, administering nicotinamide to experimental rats before STZ induction shields the pancreatic cells from the drug's damaging effects and avoids the onset of diabetes. Additionally, STZ can react with DNA's ring nitrogen and exocyclic oxygen atoms, mostly producing 7-methylguanine and 3-methyladenine, which can break DNA, activate poly-ADP-ribose polymerase, and consequently deplete NAD+.

II. Nitric oxide (NO) production

Streptozotocin's capacity to serve as a nitric oxide donor in pancreatic cells has been suggested as another potential mechanism of the diabetogenic action of the drug that causes cell death. This ability suppresses aconitase activity, which results in DNA damage and alkylation.Nitric oxide and free radicals are known to cause harm to cells, which is why streptozotocin has been demonstrated to increase the activity of guanyl cyclase and the production of cGMP, two defining activities of NO.

III. Reactive Oxygen Species (ROS) Production in Oxidative Stress

According to the definition of oxidative stress, steady state reactive oxygen species cause an imbalance in the body's pro-oxidant and antioxidant defense system. The malfunctioning of pancreatic beta-cells brought on by glucose toxicity in hyperglycemia has recently been revealed to be driven, at least in part, by oxidative stress (Baig *et al*.,2020). Both in diabetes individuals and diabetic animals, a number of reaction pathways, including as glucose auto-oxidation, protein glycation, the generation of advanced glycation products, and the polyol pathway, are thought to contribute to the development of oxidative stress. These actions result in the production of ROS, which harms tissue.

When compared to control mice, STZ administration significantly increases malonaldehyde while lowering the activity of antioxidant enzymes like catalase, glutathione peroxidase, and superoxide dismutase. Reduced antioxidant activity and concurrent increases in malonaldehyde (MDA) activity show that STZ can induce oxidative stress in the pancreas. The creation of uric acid as the end product of ATP breakdown by xanthine oxidase from hypoxanthine is a significant ROS involvement during STZ metabolism. This process speeds up the death of beta cells by producing ROS like superoxide and hydroxyl radicals from H2O2 dismutation during hypoxanthine metabolism (Alexandre – Silva *et al*., 2023).

The breakdown of beta cells is sped up by this mechanism, which produces ROS such superoxide and hydroxyl radicals from H2O2 dismutation during hypoxanthine metabolism. Along with this, glutathione peroxidase and catalase are absent from pancreatic beta cells. Subsequently, the hydrogen peroxide produces free radicals like O2- and OH-.These reactive substances have the ability to peroxidize lipids, producing hydroperoxy fatty acids and endoperoxides in the process. This stimulates the production of thromboxane-B2 (TxB2) and malonaldehyde.

Platelet aggregation and thrombosis can be facilitated by the buildup of thromboxane-A2 (TxA2) and TxB2. Aconitase, which prevents the breakdown of mitochondrial DNA (mtDNA), has also been shown to be inhibited by increased ROS generation (Haghani *et al*., 2021).

IV. Altered NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) based cell signaling

Modified NF-B-based cell signaling is a fourth molecular pathway for the cytotoxicity of STZ that causes cell death. The glycoside hydrolase O-GlcNAcase, an enzyme that cleaves beta-O-linked GlcNAc (O-GlcNAc) from modified proteins and is a member of the family 84 glycoside hydrolases, is responsible for removing O-GlcNA from proteins. STZ specifically inhibits this activity. The glycoside hydrolase O-GlcNAcase, a member of the family 84 glycoside hydrolases, is responsible for removing O-GlcNA from proteins by cleaving beta-O-linked GlcNAc (O-GlcNAc) from modified proteins (Diego *et al*., 2019). This action is especially inhibited by STZ.At lesser concentrations, STZ causes beta-cell malfunction and apoptosis, but at higher doses, it results in beta-cell necrosis.

STZ causes pancreatic beta-cell death at concentrations up to 15 mM by initiating apoptosis, which is followed by necrosis at higher levels up to 30 mM. In other biological systems, the STZ challenge (up to 20 mM) only resulted in apoptotic cell death, according to certain other studies. STZ (up to 20 mM) induces oxidative stress and apoptosis in tests conducted in vitro on insulin-secreting insulinoma cells, keratinocytes, and genetically modified hepatocytes. However, the actual molecular mechanism and metabolic targets of STZ toxicity in hepatocytes was not known. The mechanism of antineoplastic action of STZ in human hepatoma was also not clearly understood. Using the mitochondrial dehydrogenase based cellular viability MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay to investigate the dose- and time-dependent effects of STZ on human hepatoma (HepG2 cells) in culture, Haider and Annie showed that STZ induced significant cell death after 48 h of induction with 20 mM of the drug (Alexandre –Silva *et al*., 2023).

The true molecular mechanism of STZ toxicity in hepatocytes and its metabolic targets, however, were unknown. Additionally, the mechanism by which STZ acts as antineoplastic therapy in human hepatoma was unclear. Haider and Annie demonstrated that STZ significantly caused cell death after 48 h of induction with 20 mM of the drug using the mitochondrial dehydrogenase based cellular viability MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay to examine the dose- and time-dependent effects of STZ on human hepatoma (HepG2 cells) in culture. They also noted that as STZ increases the exposure to free radicals, oxidized LDL, bacterial or viral antigens, increased oxidative stress, apoptosis, and mitochondrial dysfunction in human hepatoma (HepG2) cells may be linked to altered NF-B (nuclear factor kappa-light-chain-enhancer of activated B cells, a protein complex that controls DNA transcription in all animal cell types)-based cell signaling (Sndaram *et al*.,2020).

CHAPTER THREE

MATERIALS AND METHOD

3.1 Materials

The materials used for this study are the leaf sample (*Justicia carnea*), Wistar rat, reagents and laboratory apparatus.

Apparatus

1. Beaker
2. Spatula
3. Pipette
4. Conical flask
5. Measuring cylinder
6. Separating funnel
7. Glass stirrer
8. Sieve
9. Test tubes
10. Gavage
11. Text tube rack
12. Measuring cylinder

Chemicals And Reagent

1. Streptozotocin(STZ)
2. Metformin
3. Dimethylsulfoxide (DMSO)
4. Methanol
5. Anhydrous sodium carbonate
6. Sodium hydroxide
7. Chloroform
8. Sulphuric acid
9. Acetone

10. Ferric chloride
11. Ammonia
12. Hydrochloride acid
13. Methylated spirit.

Equipment

1) Electrical weighing balance
2) Freeze drying machine
3) Spectrophotometer
4) Water bath
5) Oven
6) Glucometer
7) Centrifuge
8) Muffle furnace
9) Refrigerator

Other Materials

1. Whatman filter paper
2. Latex gloves
3. Cotton wool
4. Syringe
5. Masking tape
6. Detergents
7. Transparent buckets
8. Distilled
9 .Razor blades

3.2 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 leaves was identified and confirmed at the department of plant biology and biotechnology (PBB) University of Benin, Benin city, Edo state Nigeria.

3.2.1 Preparation of Plant Sample

The fresh leaves were thoroughly washed under running tap, air dried under shade, crushed using a mechanical grinder into a 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(3days) of soaking, the wine (dark purplish red) colored filtrate was filtered using a double cheese cloth, concentrated under reduced pressure using freeze drying machine at 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).

3.3 Experimental Animal

A total number of thirty six (36) male wistar rats (albino 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 containers for water were properly washed and rinsed with clean water. Care of experimental animals was in accordance with the Principle of Laboratory Animal Care manual guide of National Institute of Health as approved by the Institution Research Ethics committee.

3.4 Toxicity Study

Oral acute toxicity study on *Justicia carnea* methanol extract (JCME) was carried out according to Lorke method using the 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 any signs of toxicity after 60 min of administration and further observed for a period of 24 h. The absence of rat mortality in phase I necessitated the conduct of 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.

3.5 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 Streptozotocin 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*

3.5.1 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.

3.5.2 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.

3.6 Blood Glucose Test

In other to determine the blood glucose of the rats, the rats were allowed to fast overnight and the blood glucose was measured between 7-9am in the morning. The blood glucose reading was taken using a glucometer which is a device for quick determination of blood glucose level. The rats were cut at the tip of their tail using a razor blade and their blood sample was quickly collected using a glucometer test strip which was inserted back into the glucometer. The glucose reading displayed on the glucometer within 5 seconds and results were noted.

3.6.1 Working Principle of The Glucometer

The enzyme Mut.Q-GDH from *Acinetobacter calcoaceticus*, recombinant in E. coli, on the glucometer test strip transforms the glucose in the blood sample into gluconolactone. A safe DC electrical current is produced by this process, which the metre uses to determine blood glucose levels.A small AC signal is also used to assess the sample and its surroundings. The new Mut. Q-GDH enzyme was developed to eliminate maltose as a limitation, while maintaining the accuracy, precision and test strip stability of the classic PQQ enzyme system. Molecular cloning techniques were used to modify the GDH-PQQ enzyme to create the Mut. Q-GDH enzyme, which has improved molecule size discrimination. While the molecular structures of glucose and maltose are similar, the mutant enzyme can distinguish the difference in size between the two molecules thereby eliminating the maltose interference limitation.

3.7 Experiment Termination And Sacrificing The Wistar

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.

3.7.1 Homogenization

The excised liver, pancreas and 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.

3.8 Phytochemical Analysis

3.8.1 Quantitative Phytochemical Analysis of *Justicia carnea* Leaf extract

Oloruntola (2021) described and reported the methods for determining phenols, saponins, flavonoids, and tannins; while the procedures for determination of steroids were reported by Madhu et al. (2016).

1) Phenol

400 g of *Justicia carnea* Leaf extract were mixed with a total of 2000 ml of 70% ethanol, agitated for six hours, and then allowed to stand still for an additional 48 hours before filtering through Whatman No 1 filter paper. A rotary evaporator was used to vacuum condense the *Justicia carnea* Leaf methanol extract at 35–40 °C. After immersion in 1000 cc of 70% ethanol and continuous vibration for six hours, 200 g of *Justicia carnea* Leaf extract was filtered through Whatman No. 1 filter paper. The phenolic content of *Justicia carnea* Leaf extract was determined using the Folin-Ciocalteau technique, which Otles and Yalcin (2012) described. 50 mL of *Justicia carnea* Leaf extract or standard solution were combined with 250 mL of the Folin-Ciocalteau reaction. For five minutes, this mixture was left at ambient temperature in a dim environment. 750 microliters of a 7 percent Na2CO3 solution were added at the end of the time period. The mixture was diluted with distilled water to 5 mL. After that, the mixture was allowed to react for 120 minutes at room temperature in a dim setting. Both the standards and the samples' absorbance were calculated at 760 nm. The blank solution received 50 l of an 80 percent methanol solution in place of the 50 l of extract. A calibration curve was utilized to calculate the total phenolic content using standards that are equivalent to gallic acid.

1) Alkaloids

The gravimetric method was used to determine the alkaloid content of the leaf sample (Adeniyi *et al.*, 2009). 5 g of the *Justicia carnea* Leaf extract were mixed with 50 ml of a 10% w/v acetic acid solution in ethanol. The mixture was vibrated and left alone for around 240 minutes before being sieved. The filtrate was diluted to one-fourth of its initial volume on a hot plate. The alkaloids were then precipitated by adding Droplets of very concentrated ammonium hydroxide. The precipitate was first washed with a 1% solution of ammonium hydroxide before being filtered through filter paper. The precipitate was then transferred to desiccators, where it was dried in an oven for 30 minutes at 60°C before being weighed again until it reached a constant weight. Alkaloids' mass expressed as a percentage.

2) Saponins

According to Adeniyi *et al.*, (2009), the vanillin and concentrated sulfuric acid colourimetric method was used to measure saponin. 0.5 ml of 50% ethanol, 4.0 ml of 77 percent sulfuric acid, and 0.5 ml of freshly prepared vanillin solution were added to the 0.1 ml of *Justicia carnea* Leaf extract. After being allowed to cool to room temperature, the mixture was heated in a water bath for 15 minutes to 60 °C. A UV/Vis spectrophotometer was used to detect the absorbance at 545 nm. The total amount of saponin in each sample was determined using a tea saponin calibration curve and represented as mg tea saponin equivalent per g (TSE/g DW).

3) Steroids

Steroid concentration in *Justicia carnea* Leaf extract was found, according to Madhu et al.'s (2016) studies. According to Madhu et al. (2016), steroids were identified. 1 ml of *Justicia carnea* Leaf steroid extract was placed in 10 ml volumetric flasks. Sulphuric acid (4N, 2 ml), iron (III) chloride (0.5 percent w/v), and potassium hexacyanoferrate (III) solution were then added. Before being diluted with distilled water to the proper concentration, the mixture was heated for 30 minutes at 70-20 °C in a water bath with intermittent shaking. The absorbance was calculated at 780 nm in relation to a reagent blank.

4) Flavonoids

Flavonoids in *Justicia carnea* Leaf extract were analyzed for concentration based on Surana *et al* (2016) methodology, 2.8 ml of distilled water, 0.1 ml of potassium acetate solution, 1.50 ml of methanol, and 0.1 ml of aluminum chloride solution A test tube holding 0.50 ml of *Justicia carnea* Leaf extract received water additions. The same method, but with distilled water instead of aluminum chloride solution, was used to make sample blanks for extract and rutin standard dilutions (10-100 g/ml). After that, Whatman filter paper (No. 1) was used to filter the solutions. Absorbance ratios were measured at 510 nm in contrast to blanks. The overall flavonoid content was then discovered to be equal to 1 mg of rutin per gram of the methanol *Justicia carnea* Leaf extract. The Folin-Ciocalteau method was used to calculate the total tannin concentration (Biswas *et al*., 2020). A volumetric flask (100 ml) was used to dilute 1 ml of the *Justicia carnea* Leaf methanol extract with 49 ml of distilled water, 1.7 ml of 75% ethanol, 0.1 ml of metaphosphoric acid, 10 ml of 1.0 mol/ml Na2CO3, and 2.5 ml Folin-Ciocalteu. The mixture was thoroughly blended and then let to cool for 15 minutes at room temperature. Then, using a spectrophotometer, the absorbance of the standard solution and *Justicia carnea* Leaf extract was determined at 680 nm in comparison to a control. To express the sample's total tannin content as a reference against the standard curve, tannic acid (TA) mg TA/g dry weight was used (R2 = 0.9972).

3.9 Determination Of Antioxidant In *Justicia carnea*

3.9.1 DETERMINATION OF CATALASE (CAT)

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

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

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

3.9.2 ESTIMATION OF SUPEROXIDE DISMUTASE ACTVITY (SOD)

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

3.9.3 ESTIMATION 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

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

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

3.9.4 DETERMINATION OF MALONDIALDEHYDE (MDA)

Malonaldehyde was determined using the thiobarbituric acid assay (Buege and Aust, 1978)

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;

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

A= Absorbance

V= Total volume of reaction mixture

M= Molar extinction coefficient

V= volume of the sample

Y= mg protein

3.10 Total Protein Assay

Principle: protein molecules are made up of amino acids which are arranged in long chains called peptide chains, and the links joining the amino acids together are called peptide bonds.

The biuret method is based on the reaction which occurs between cupric ions in the regents and peptide bonds of the protein molecules in an alkaline solution to form blue violet or purpose colored complex. The absorbance of the color is measured in a spectrophotometer at 540nm.

Reagents: Biuret reagent (stock solution), biuret working solution, tartrate iodide solution, standard protein.

Procedure for test:

	Test	Test blank	Standard	Standard blank	Blank
Biuret reagents	5.0ml	-	5.0ml	-	5.0ml
Tartanate iodide	-	5.0ml	-	5.0ml	-
Serum	0.05ml	0.05ml	-	-	-
Standard	-	-	0.05ml	0.05ml	-
Distilled	-	-	-	-	0.05ml

Table 3.1

The above table shows the procedure used for estimation of serum total protein.

i. Five test tubes were set up and reagents added as shown above.
ii. It was mixed and allowed to stand at room temperature for 10mins
iii. The absorbance was read at 540nm

Calculation:

Gram of serum protein/dl = $\frac{Ab.of\ test - Ab.test.blank}{Ab.\ of\ test - Ab.\ std.blank}$ x conc of std.

3.11 liver And Kidney Function Test:

3.11.1 ESTIMATION OF TOTAL AND CONJUGATED SERUM BILIRUBIN

Background: Bilirubin is a yellow pigment that occurs in the normal catabolic pathway. Catabolism is a necessary process in the body clearance of bye-products that arise from the destruction of aged red blood cells. First, the hemoglobin gets stripped of the heme (iron) molecule which thereafter, passes through various processes of porphyrin catabolism; depending on the part of the body which the break down takes place. For examples, the molecules extracted in the urine differ from those in the feces. Bilirubin is called "conjugated" or "direct" bilirubin. Bilirubin is conjugated when it contain glucoronic acid which is acted upon by the enzyme, glucuronyl transferase, making it soluble in water).

Reagents: sample (serum/plasma), sulphanillic acid reagent, sodium nitrate, solution, Diazo reagent, caffeine reagent, Alkaline tartarate reagent, Ascorbic acid solution, standard bilirubin solution.

Procedure:

Five (5) test tubes were set up and reagents added as shown in the table below:

	Total bilirubin(TB)	Conjugated bilirubin(CB)	Blank(B)	Standard(SD)	Standard blank(SB)
Serum	0.2ml	0.2ml	-	-	-
Standard(200µmol/L)	-	-	-	0.2ml	-
Distilled water	0.8ml	0.8ml	0.8ml	0.8ml	0.8ml
Diazo reagent	0.5ml	0.5ml	-	0.5ml	-
Caffeine reagent	0.2ml	-	-	2.0ml	-
Ascorbic acid	0.1ml	0.1ml	0.1ml	0.1ml	0.1ml
Diazo reagent	-	-	0.5ml	-	0.5ml
Serum	-	-	0.2ml	-	-
Standard(200µmol/L)	-	-	-	-	0.2ml
Caffeine reagent	-	2.0ml	2.0ml	-	2.0ml
Alkaline tartarate	1.5ml	1.5ml	1.5ml	1.5ml	1.5ml

Table 3.2

Incubated at room temperature for 10mins

The above table shows the procedure used to measure the amount of total and conjugated bilirubin.

The absorbance of the tubes was read at 600nm within 30mins.

Calculation:

Total bilirubin (TB) umol/L= $\frac{Absor.(TB)-Absorbance\ (B)}{Absorb.\ (s)-Ab.\ (SB)}$x 200

Conjugated Bil. (CB) umol/L=$\frac{Absor.(CB)-Ab.\ (B)}{Absorb.\ (s)-Ab.\ (SB)}$x 200

Bil. Umol/L = mg/dl

3.11.2 ESTIMATION OF SERUM ASPARTATE AMINOTRANSFERASE (AST)

Background: Aspartate aminotransferase (AST) is a cellular enzyme present in many tissues such as heart, skeletal muscles, kidney, brain, liver, pancreases or erythrocytes. It exists in two isoforms, cytoplasmic and mitochondrial. The cytoplasmic isoenzymes are released into the blood during the moderate cell damage. One the other hand, the activity of the mitochondrial isoenzymes in blood increases during the severe cell damage. The determination of AST activity in serum is used mainly to assess the liver damage.

Principle:

L-Aspartate – 2 ketoglutarate – AST → Oxaloacetate + L – glutamate

Oxaloacetate+ 2, 4 dinitropheny/hydrazine + Alkaline 2, 4dinitrophenyl/hydrazine (red brown).

When aspartate aminotransferase (AST) is unabated at 37^0C for exactly 60mins in a pH 7.5 buttered substrate containing aspartate and 2 – ketoglutarate, it catalyzes the transfer or amino acid group from aspartate to ketoglutarate, forming oxaloacetate, and glutamate. The oxaloacetate react with 2,4-dinitrophenyl/ hydrazine to form 2, 4 dinitropheny/hydrazine which in alkaline P^H is red brown. The absorbance of the colors is measured in the spectrophotometer at 505nm.

Reagent: Phosphate butter (pH 7.4 01m) substrate for AST, dinitrophenyl/hydrazine (DNPH) reagent, 0.4N sodium hydroxide, pyruvatested.

Procedure for test:

Test tubes were set up as shown in the table below:

	Test	Control
Buffered substrate	0.5ml	0.5ml
	Incubated at 37^0C to 3mins	
Serum	0.1ml	-
Serum	Incubated at 37^0C to 60mins -	0.1ml
DNPH	0.5ml	0.5ml
0.4NOH	Incubated at 37^0C to 20mins 5.0ml	5.0ml

Table 3.3

The above table shows the procedure for estimation of serum aspartate aminotransferase.

(ii) It was allowed to stand at room temperature for 5-10mins and the absorbance was read at 505nm.

3.11.3 ESTIMATION OF SERUM ALANINE AMINOTRANSFERASE (ALT).

Background: Alanine aminotransferase (ALT) is a cytoplasm enzyme. It is basically localized in hepatocytes. It is basically localized in hepatocytes. It is released into the blood during the cell damage.

Principle:

Alanine + α-Ketoglutarate →Pyruvate + L-Glutamate

Pyruvate + DNPH → Pyruvate +Dinitrophenylhydrazone.

ALT catalyzes the transfer of amino group between L-alanine and L-glutamate; the corresponding α-keto acid in this process is α-ketoglutarate and Pyruvate, forming Oxaloacetate and glutamate.

Test procedure:

	Test	Control
Buffered substrate	0.5ml	0.5ml

Incubate at 37^{o}C for 3mins.

Serum	0.1ml	-

Table 3.4

Incubate at 37^{o}C for 30mins

Serum	-	0.1ml
DNPH	0.5ml	0.5ml

Incubated at 37^{o}C for 20mins.

0.4N NaOH	5.0ml	5.0ml

Table 3.5

The above table shows the procedure for estimation of serum Alanine Aminotransferase.

i. Test tubes were set up as shown in the table above:
ii. The mixture was allowed to stand at room temperature for 5-10mins and the absorbance was read at 505nm.

3.11.4 ESTIMATION OF SERUM ALBUMIN

Principle: Under acidic condition, serum Albumin binds specifically with Bromocresol green to form a green colored complex.

Reagents: Bromocresol green reagent, Albumin standard (4%), blank reagent.

Procedure for test:

The below table shows the procedure for the estimation of serum albumin.

	Test	Standard	Blank
BCG reagent	5.0ml	5.0ml	-
Blank reagent	-	-	5.0ml
Serum	0.05ml	-	-
Standard	-	0.05ml	-
Distilled water	-	-	0.05ml

Table 3.6

i. Three (3) test tubes were set up and reagent added as shown above:
ii. It was mixed well and allowed to stand at room temperature for 10mins
iii. The absorbance was read at 640nm

Calculation:

Gram of serum albumin/dl = = $\frac{Ab.\ test}{Abs.\ std}$ x conc. of std= $\frac{Ab.\ test}{Abs.\ std}$ x 4

3.11.5 ESTIMATION OF PLASMA UREA.

Background: Urea constitutes almost half the total of the non-protein nitrogenous substances in the blood. It is produced in the liver from carbon dioxide and ammonia resulting from the breakdown of amino acid. It is the major product of the excretory system of protein metabolism. Urea is carried by the kidney where it is filtered from the plasma in the glomerulus. About 40% of the urea glomerular filtrate is reabsorbed by the renal tubules. Most of the urea in the filtrate is extracted in the urine while small amount is extracted through the gastrointestinal tract and the skin.

Principle: In the method, ammonia generated by the action of an enzyme, urease on urea is estimated by measuring the blue color of the indophenol formed with the phenol and hypochlorite. Sodium nitroprusside is used as a catalyst.

Reagents: Buffered urease reagent, phenol color reagent, alkaline hypochlorite reagent, urea standard solution (10mmol/l).

Test procedure.

	Blank(µl)	Test(µl)	Standard(µl)
Buffered urease reagent	200	200	200
Test plasma	-	20	-
Standard	-	-	20
Distilled water	20	-	-

Table 3.7

The above table shows the estimation of serum urea.

i. Test tubes were set up and reagent added as shown in the table above
ii. The test tubes were incubated at 37^{o}C for 15mins in a water bath.
iii. 1.0ml of phenol reagent was added to each tube and shaken gently to mix.
iv. 1ml hypochlorite reagent was added to each tube and mix.
v. The test tubes were incubated at 37^{o}C for 20mins in the water bath.
vi. 5ml of distilled water was added to each tube.
vii. The absorbance was read against the standard at 630-700nm.

Calculations:

The plasma urea concentration in mmol/l $= \frac{Absorbance\ of\ test}{Absobance\ of\ std} \times$ 10mml/L

3.11.6 ESTIMATION OF SERUM URIC ACID.

Background: Uric acid is the final product of the purine metabolism. Purines such as adenosine and guanine result from the breakdown of nucleic acids. They are either ingested or results from the destruction of tissue cells, and are converted mainly in the liver into the uric acid. From the liver, uric acid is transported to the kidneys where it is filtered by the glomeruli.

Nearly, all the uric acid is reabsorbed in the proximal tubules and small amount are then secreted by the distal tubules and ultimately appear in the urine. Nearly all the uric acid in the plasma is in the form of monosodium urate. Urate in this form is relatively insoluble. At high level (>6.4mg/dl), the plasma is saturated; urate crystals may form and participate in the tissues.

Principle: Uric acid is readily oxidized to Allantoin and so can function as reducing agent in the many chemical reactions. The Caraway method is the most popular method for determining uric acid level. This method is based on the oxidation of uric acid in a protein free solution, with eventual reduction of phosphotungstic acid to the tungsten blue. Sodium carbonate is used to provide the alkaline P^H necessary for color development. Unfortunately, this method is relatively non-specific, and so, several methods that make use of the specific enzyme, uricase have been developed. This enzyme catalyzes the oxidation of the uric acid to the Allantoin with the subsequent production of hydrogen peroxide (H_2O_2).

The uricase method is based on the fact that, uric acid has a significant UV absorbance with a peak at 293nm, whereas Allantoin does not have.

Reagents: Phosphotungstic acid reagent, sodium carbonate solution, uric acid standard(stock) 50mg/dl, uric acid standard (working) 5mg/dl.

Procedure:

	Test	Standard	Blank
Phosphotungstic acid reagent	2.0ml	2.0ml	2.0ml
Sample	0.2ml	-	-
Working standard uric acid(5mg/dl)	-	0.2ml	-
Distilled water	-	-	0.2ml

Table 3.8

i. Three (3) test tubes were set up and reagents added as shown above.
ii. The mixture was mixed and allowed to stand at room temperature for 15mins.
iii. All the tubes was centrifuged at 3000RPM for 10mins
iv. The supernatant was decanted directly into 3 separate correspondently labeled tubes.
v. 1.0ml of sodium carbonate solution was added
vi. The mixture was allowed at room temperature for 15mins and the absorbance was read at 650-700nm.

Calculations:

$$\text{Serum uric acid in mg/dl} = \frac{\text{Absorbance of test}}{\text{Absorbance of std}} \times 5$$

Serum uric acid mg/dl × 0.059 = mmol/L

3.11.7 ALKALINE PHOSPHATASE

ReagentComposition:

1. Alkaline phophastase Substrate: 3.6mM,Sodium ThymolpthaleinMonophosphate In 0.2M 2-Methyl-1-Propanol buffer. Magnesium Chloride 1.0mM, weting agent, in active ingredients, preservatives; pH10.2.
2. Alkaline Phosphastase Color Developer: 0.1M Sodium Hydroxide, 0.1M sodium Carbonate.

3. Alkaline Phosphastase Standard: Thymolpthaleininn propanol 0.5mM/L. Equivalent to 50U\L enzyme activity when used according to the Alkaline Phosphastase.

Principle:

The alkaline phosphatase acts upon AMP–buffered sodium thymolphthalein monophosphate. The addition of an alkaline reagent stops enzyme activity and simultaneously develops a blue chromogen, which is measured photometrically.

Procedure:

1. For each sample, dispense 0.5ml of alkaline phosphatase substrate into labeled test tubes and equilibrate to 37oC for three (3) minutes.
2. At timed interval (1minute) add 0.005ml of each standard, control, and sample to its respective test tube. Mix gently. Use deionized water as sample for reagent blank.
3. Incubate for exactly ten (10) minutes at 37oC.
4. Following the same sequence as in step2, add 2.5ml alkaline phosphatase color developer at timed intervals. Mix well.
5. Set the wave length of the spectrophotometer at 590nm. Zero with reagent blank.
6. Read and record absorbance of samples.

Calculation:

Absorbance of unknown x Value (concentration) of Std. (IU/L) = concentration of ALP (IU/L)

3.12 Lipid Profile Assay

3.12.1 ESTIMATION OF SERUM/PLASMA CHOLESTEROL

Principle: In the presence of excess acid such as phosphoric acid and ferric (fe3+) ions, cholesterol is oxidized to disulphonic and which is reddish purple in color. It is read spectrophotometrically at 560mm against standard and a blank.

Reagents: ferric chloride reagents, cholesterol standard, serum/plasma (after 12hrs of fasting).

Procedure for test:

	Test	Standard	Blank
Ferric chloride reagent	5.0ml	5.0ml	5.0ml
Diluted serving	0.5ml	-	-
Diluted standard	-	0.5ml	-
Diluted water	-	-	0.5ml

Table 3.9

The above table shows the procedure for carrying out cholesterol estimation.

i. The serum was diluted 1:20 with distilled water (0.1m1 of serum + 1.9ml of distilled water).
ii. The cholesterol standard was diluted at 1:20 with glacial acetic acid.
iii. Three (3) tubes was set up as shown in the below table.
iv. Each test tube was shake for 10secs to mix the content
v. The test tubes were immediately place in a booking water bath for 90secs.
vi. It was allowed to cool in running tap water for 5mins.
vii. The absorbance was read at 560mm against the blank and standard.

Calculation:

Serum cholesterol (mg/dl) = $\frac{Abs\ of\ test}{Abs\ of\ standard} \times 250$

Serum cholesterol (mol 11) = mg/dl 0.0259

3.12.2 PLASMA TRIACYLGLYCEROL:

Principle:

Lipoprotein lipase hydrolyses triglycerides to glycerol and free fatty acids. The glycerol formed reacts with ATP in the presence of glycerol kinase to form glycerol-3-phosphate, which is oxidized to form hydrogen peroxide by the enzyme-glycerolphosphateoxidase. The hydrogen peroxide reacts with 4-aminophenazone to form a quinoneimine dye.

$$\text{Triglycerides} + H_2O \xrightarrow{\text{Lipases}} \text{Glycerol} + \text{Fatty acids}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{GlycerolKinase}} \text{glycerol-3-phosphate} + \text{ADP}$$

$$\text{Glycerol-3-phosphate} + O_2 \xrightarrow{\text{Glycerol-3-phosphateoxidase}} \text{Dihydroxyacetonephosphate} + H_2O_2$$

$$2H_2O_2 + \text{4-aminophenazone} + \text{4-chlorophenol} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine} + HCl + 4H_2O$$

Reagent Composition:

R1a. Buffer

Contents	Initial concentration of solutions
Pipes buffer	40mmol/L, pH7.6
4–chlorophenol	5.5mmol/L
Magnesium ions	17.5mmol/L

Table 3.10

R1b. Enzyme Reagent

Contents	Initial concentration of solutions
4–aminophenazone	0.5mmol/L
ATP	1.0mmol/L
Lipases	≥150U/ml
Glycerol-kinase	≥0.4U/ml
glycerol–3phosphateoxidase	≥1.5U/ml
Peroxidase	≥0.5U/ml
CAL. Standard	lot specific insert

Table 3.11

Procedure:

The following were pipetted into separate clean dry test tubes labeled as blank, standard and test.

Addition sequence	Blank(µl)	Standard(µl)	Sample(µl)
Dist. H_2O	10	-	-
Sample	-	-	10
Standard	-	10	-
Reagent	1000	1000	1000

Table 3.12

The solutions were mixed and incubated for 5 minutes at 37^0C. The absorbance readings of the sample and standard were measured against the blank reagent within 60 minutes, at wavelength of 500nm.

Calculations:

Triglyceride concentration = $\frac{A_{sample}}{A_{standard}} \times$ standard conc.(mmol/l) = mmol/l

$= \frac{A_{sample}}{A_{standard}} \times$ standard conc.(mg/dl) =mg/dl

3.13 Statistical Analysis

The statistical tool SPSS (Statistical Package for the Social Sciences) version 7 was used for data analysis. Data was presented as mean standard deviation. Analysis of variance in one direction (ANOVA) was used to examine the statistical significance of the results between groups. At $P<0.05$, differences between means were deemed significant.

CHAPTER FOUR

RESULTS

4.1 Weight Of The Rats

GROUPS	INITIAL BODY WEIGHT ($g\times10^{-3}$)	FINAL BODY WEIGHT ($g\times10^{-3}$)	CHANGE IN BODY WEIGHT ($g\times10^{-3}$)
Group 1 (Control)	109.53±3.13	192.80±14.34	83.28±14.49
Group 2 (Diabetic Control)	212.11±14.41	133.75±17.24	78.36±9.15
Group 3 (Metformin)	178.58±5.23	118.33±11.19	66.20±9.74
Group 4 (100mg of Extract)	176.67±12.64	118.36±18.24	58.31±9.52
Group 5 (200mg of Extract)	112.99±4.43	153.04±5.54	41.95±1.45
Group 6 (500mg of Extract)	211.08±6.59	125.81±15.88	85.27±9.29

Table 4.1

4.2 Result Of Acute Toxicity

Results of phase 1 of acute toxicity studies

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

Table 4.2

Results of phase 2 of acute toxicity studies

Dose(mg/kg)	Mortality
1600	0/3
2900	0/3
5000	0/3

Table 4.3

4.3 Phytochemical Analysis

S/N	PHYTOCHEMICAL COMPONENTS	REMARK
1	FLAVONOIDS	++
2	TANNINS	++
3	CARDIAC GLYCOSIDES	-
4	SAPONIN	-
5	STEROIDS	++
6	PHENOLS	++
7	PHLABOTANNINS	-
8	COUMARIN	-
9	ALKALOIDS	++
10	ANTHRAQUINONE	-
11	TERPENOIDS	++

Table 4.4

KEY:

- Negative (Absent)

+ Positive (Present) but low

++ (High)

+++ (very high)

4.4 Result Of Antioxidant Activity

Invivo Antioxidants Analysis (Pancreas)

ACTIVITY	CONTROL	GROUP 1	GROUP 2	GROUP 3	GROUP 4	GROUP 6	F	Sig.
SOD (unit/mg protein)	0.980±0.098	1.107±0.00	1.273±0.00	1.137±0.080	1.651±0.00*	2.659±0.00*	9.41	0.00
CAT (unit/mg protein)	0.370±0.068	0.072±0.00	0.466±0.057	0.485±0.056	0.820±0.00*	0.948±0.00*	5.75	0.01
GPx (u/L)	2.589±0.349	1.774±0.00	3.320±0.597	3.319±0.411	5.711±0.00*	6.536±0.00*	5.55	0.01
MDA (unit/mg protein)	1.503±0.146	1.333±0.00	1.659±0.421	1.863±0.144	1.235±0.00	4.179±0.00*	38.81	0.00

Table 4.5

Values are expressed as Mean ± SEM.

Values with superscript show significant difference from the control

4.5 Blood Glucose Reading

Below is a result of the effect of *Justicia carnea* methanol extract on the blood glucose of Streptozotocin induced diabetes wistar rat

	INITIAL FBS (22/07/23)	FBS WHEN INDUCED (25/07/23)	FBS AFTER TREATMENT (29/07/23)	FBS AFTER TREATMENT (05/08/23)	FBS AFTER TREATMENT (12/08/23)
GROUP 1	76.00±4.15^{a}	_	103.67±2.53^{a}	92.33±2.37^{a}	65.5±3.42^{a}
GROUP 2	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}
GROUP 3	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}
GROUP 4	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}
GROUP 5	56.80±1.65^{a}	370.20±49.42^{a}	321.75±53.10^{c}	295±112.155^{d}	222.33±62.22^{c}
GROUP 6	59.17±6.32^{a}	297.67±43.31^{a}	240.20±70.83^{c}	190.00±55.08^{c}	53.00±12^{a}

Table 4.6

Values are expressed as Mean ± SEM.

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

Table 4.6 shows the blood glucose level determined before diabetes induction, 48 hours after induction, 7 days after first treatment and day 14 after first treatment. Within 48hrs after induction of diabetes, there was increase in blood glucose level in all the rats as shown on day 2. After treatment there was a significant decrease in blood glucose level in diabetic treated groups (groups 3, 4, 5and 6) when compared with diabetic untreated group 2 as shown on day 14.

4.6 Result of Lipid Profile Test

Below is a result of the effect of *Justicia carnea* methanol extract on the lipid profile of Streptozotocin induced diabetes wistar rat

	GRP 1	GRP 2	GRP 3	GRP 4	GRP 5	GRP 6
LDL	291.62±64.34	498.91±27.74*	419.36±32.89	200.69±52.60*	168.76±16.45*	95.78±7.90*
TRIG	2.87±0.02	2.58±0.16	2.37±0.02	2.32±0.01	2.34±0.03	2.30±0.01
HDL	187.58±28.76	106.02±35.37*	83.59±13.37*	212.04±14.70	275.71±23.51*	289.43±7.35*
CHOL	88.49±0.72	149.64±1.44*	93.52±1.44	95.68±2.16	53.96±0.72*	77.70±4.32*

Table 4.7

Values are expressed as mean ± SEM.

*. The mean difference is significant at the 0.05 level.

The effects of *Justicia carnea* leaf extract in ethanol on the lipid profile of diabetic rats as compared to control rats are shown in Table 4.7. Total cholesterol (TC) levels were substantially higher in the untreated diabetic control group (group 2) than in the normal control group (group 1) ($p >0.05$). When compared to the untreated diabetic control group (2), group 5 treated with 200mg of the extract showed the greatest reduction in serum levels of total cholesterol ($p<0.05$) across all doses. There was no statistically significant difference between the Metformin-treated group (3) and the 100 mg/kg extract-treated group (4) and the normal group (1) ($p<0.05$). When compared to the untreated diabetic control group (2), there was a non-significant increase ($p<0.05$) in the group treated with 100mg/kg of the extract but a significant decrease ($p>0.05$) in the serum triglyceride (TG) in the group treated with 200mg/kg of the extract. There was no statistically significant difference between the methformin-treated group and the 100mg/kg extract-treated group and the normal group ($p>0.05$) in either group (1).

There was a significant decrease (P>0.05) in the serum triglyceride in the group treated with *Justicia carnea* extract (group 4,5 & 6) when compared with normal control group (1). There was a statistical significant decrease in the group treated with the standard drug methformin.

The high density lipoprotein decreased significantly in the 200kg/mg and 500kg/mg extract treated group and metformin treated group (3) when compared with normal treated group (1).

Between 200kg/mg, 500kg/mg *Justicia carnea* extract treated group and metformin treated group (3), there was no discernible change in their triglyceralide (P< 0.05).

4.7 Result of Kidney Function Test

Below is a result of the effect of *Justicia carnea* methanol extract on the kidney function of Streptozotocin induced diabetes wistar rat

	GRP 1	GRP 2	GRP 3	GRP 4	GRP 5	GRP 6
CREATINE	0.21±0.03	1.18±0.01*	0.15±0.03	0.22±0.06	0.19±0.02	0.14±0.03*
CHLORIDE	93.30±0.55	93.57±8.31	98.48±5.03	91.78±3.92	99.82±6.94	91.51±3.25
SODIUM	140.61±3.97	124.24±4.49*	130.42±1.19*	130.91±1.31*	135.15±4.28	131.76±0.32
POTASIUM	3.91±0.11	4.23±0.29	4.32±0.31	4.75±0.23	4.14±0.25	3.8±0.07
UREA	39.43±0.31	48.97±3.39	45.43±0.15	30.08±0.30	41.07±9.24	48.31±0.56

Table 4.8

Values are expressed as mean ± SEM.

*. The mean difference is significant at the 0.05 level.

Table 4.8 displays the serum concentrations of urea and creatinine in normal control, diabetic rats treated with Methformin, diabetic rats left untreated, and diabetic rats treated with 100, 200 and 500 mg/kg of *Justicia carnea* methanol extract, respectively. In compared to Groups 1 and 2 (the normal and untreated diabetes control), the urea concentrations were considerably higher (P< 0.05) in the treated groups except the group treated with 100kg/mg *Justicia carnea* extract. Although the serum creatinine concentration after treatment with *Justicia carnea* extract was non-significantly lower (P<0.05) than it was in the normal control group, it was considerably higher (P<0.05) in group 2 (the untreated diabetic control). Although the serum creatinine concentration after treatment with *Justicia carnea* extract was non-significantly lower (P>0.05) than it was in the normal control group, it was considerably higher (P<0.05) in group 2 (the untreated diabetic control).

4.8 Result of Total Protein Assay Test

Below is a result of the effect of *Justicia carnea* methanol extract on the total protein of Streptozotocin induced diabetes wistar rat

	GRP 1	GRP 2	GRP 3	GRP 4	GRP 5	GRP 6
TOTAL PROTEIN	6.26±0.68	3.96±0.51*	6.46±0.15*	5.83±0.16*	5.82±0.34*	5.40±0.16*
ALBUMIN	3.07±0.18	2.61±0.25	2.19±0.24*	2.34±0.27	1.89±0.37*	1.95±0.21*
T/BILIRUBIN	0.09±0.01	1.70±0.48*	0.54±0.11	0.55±0.13	0.62±0.08	0.25±0.02
D/BILIRUBIN	0.19±0.04	0.61±0.17	0.73±0.52	1.10±0.31*	1.06±0.2*	0.43±0.03

Table 4.9

*. The mean difference is significant at the 0.05 level.

When compared to the normal control group, the level of total protein in the diabetic untreated group was significantly low (P >0.05) after the induction of diabetes in rats. Both the extract and the common medications significantly (p >0.05) increase the total protein in diabetic rats.

4.9 Result of Liver Function Test

Below is a result of the effect of *Justicia carnea* methanol extract on the liver function of Streptozotocin induced diabetes wistar rat

	GRP 1	**GRP 2**	**GRP 3**	**GRP 4**	**GRP 5**	**GRP 6**
TOTAL PROTEIN	6.26±0.68	3.96±0.51*	6.46±0.15*	5.83±0.16*	5.82±0.34*	5.40±0.16*
ALP	0.035±0.01	0.168±0.04*	0.121±0.122	0.219±0.06*	0.214±0.04*	0.254±0.01
ALT	275.16±3.59	357.27±3.34*	272.69±1.11	281.59±4.84	255.99±8.03*	276.35±4.77
AST	281.59±1.11	388.44±6.20*	271.61±6.77	291.60±5.55	253.76±10.73*	269.63±2.85
ALBUMIN	3.07±0.18	2.61±0.25	2.19±0.24*	2.34±0.27	1.89±0.37*	1.95±0.21*
T/BILIRUBIN	0.09±0.01	1.70±0.48*	0.54±0.11	0.55±0.13	0.62±0.08	0.25±0.02
D/BILIRUBIN	0.19±0.04	0.61±0.17	0.73±0.52	1.10±0.31*	1.06±0.2*	0.43±0.03

Table 4.10

Table 4.10 compares the effects of administering *Justicia carnea* leaf extract to the control group and the Streptozotocin induced diabetic rat for liver enzymes. The AST (aspartate transaminase) and

ALT (alanine transaminase) of the diabetes untreated group (group 2) significantly increased ($p<0.05$). In groups 4,5 and 6, the administration of *Justicia carnea* leaf extract in ethanol at doses of 100 mg/kg, 200 mg/kg, and 500 mg/kg, respectively, resulted in a substantial reduction ($P <0.05$), much like the normal control group (1) and the diabetic group (3), which is being treated with the usual medication metformin. The highest levels of ALT and AST lowering were seen at doses of 200 mg/kg and 500 mg/kg, respectively. There was no discernible difference between the normal control group and the diabetic treated with methformin treatment group. Alkaline phosphatase, or ALP, was also considerably elevated ($p>0.05$) in the extract-treated groups (4 &5) and in the diabetic group receiving the standard treatment compared to the normal control group.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

According to the results of the acute toxicity (LD50) test, the extract's acute oral LD50 in rats was greater than 5000mg/kg, this indicates a large margin of safety. The results of the fasting blood glucose test indicated that the administration of the leaf extract to the rats reduced their blood glucose levels. By depriving the animal of insulin, the pancreatic beta cell is damaged through Streptozotocin-mediated oxidative damage, causing diabetes mellitus (Nwodo *et al*.,2015). *Justicia carnea's* capacity to treat and reverse diabetes brought on by Streptozotocin can also treat side effects brought on by the pancreas beta -cell degeneration.

In this research, it was found out that increased production from the injured pancreatic cells brought on by the injection of Streptozotocin may be the reason of the considerable increase in blood urea and creatinine levels seen in the diabetes groups as compared to the healthy control group. Increased plasma circulation of free glucogenic amino acids, which are then deaminated in the liver and result in a rise in urea. Urea is produced as a byproduct of the breakdown of proteins and amino acids which is made in the liver from ammonia during the deamination of proteins (Suryawanshi *et al*.,2014). Comparing the group of diabetic animals given the leaf extracts to the healthy control, serum urea level also rose significantly ($p>0.05$) in the group of diabetic animals. Therefore, this increase may be the result of Streptozotocin-induced pancreatic cell damage that was not repaired after administration of the extract. The amount of creatinine in serum is thought to be proportional to the body's muscle mass. Creatinine is a byproduct of muscle creatine (Singh *et al*., 2012). Since creatinine is constantly present in the same amount and is quickly eliminated by the kidneys, higher amounts suggest decreased renal function. Increased levels of creatinine indicate that more creatinine was kept in the blood as seen in the diabetic group (2) who were not receiving treatment.

However, as seen in groups 4,5 and 6, the creatinine level dramatically decreased ($p<0.05$) when the extracts were administered. A higher dose of the leaf extracts provided a greater effect (lessened creatinine retention), which was dose dependant. This suggests that the leaf extracts offer protection against renal impairment brought on by diabetes problems.

In the current study, rats with diabetes had higher lipid profiles. The serum levels of low-density lipoprotein (LDL), and total cholesterol (TC) were decreased after administration of 100 mg/kg ethanol leaf extract of *Justicia carnea*, whereas high density lipoprotein was increased. The effectiveness of the extract is thus observed to be dose-dependent; however, at 200mg/kg and 500mg/mg dose, the serum levels of LDL, T 70 d TC were decreased while the serum levels of HDL were increased. This hypolipidemic activities shown by the leaf extract might be due to ability of extract to cause regeneration of the beta-cells of the pancreas and potentiation of insulin secretion from surviving beta cells. The elevated blood HDL levels that were seen in the animals at a dose of 100mg/kg, 200 mg/kg and 500mg/kg may have been a result of the extract's ability to lower cholesterol levels. A little over 30% of blood cholesterol is carried by HDL. A highly ideal biochemical condition for the prevention of atherosclerosis and ischemic situations is a significant decrease in total cholesterol and an increase in HDL. High levels of HDL protect against cardiovascular disease by removing cholesterol antheroma from arteries and transporting it back to the liver for excretion or reutilization. The plant extract may also be helpful in managing hyperlipidemia and hypercholesterolemia complications, which frequently coexist with diabetes. Furthermore, the stabilization of serum TG and TC levels in rats by the plant extract may be attributed to glucose utilization and thus depressed fat mobilization (Singh *et al*., 2012 & Nkosi 2015).

Electrolyte imbalance in diabetics is a typical complication brought on by changed distribution of electrolytes. It is also connected to osmotic fluid shifts brought on by hyperglycemia or total body deficits caused by osmotic diuresis (Nwodo *et al.*, 2015). A healthy balance of electrolytes in the blood is a sign that the kidneys and heart are working properly. Dysnatremias are brought on by diabetes mellitus through a number of underlying causes. Since glucose is one of the osmotically active substances, diabetes forces an osmotic diuresis, which has the side effect of electrolyte loss due to dehydration. This will raise the serum's osmolality in hyperglycemia, which causes water to flow out of the cells. Osmotic diuresis caused by uncontrolled hyperglycemia also causes hypovolumichyponatremia (Singh *et al*., 2012). According to this study, diabetes had an impact on the animals' serum electrolytes, just as it had on the diabetics group who weren't receiving treatment. When compared to the normal control group, it was shown that the untreated diabetes groups' serum electrolyte levels rose. The restoration of the altered electrolytes following delivery of the leaf extracts shows that the extract protected the kidneys from damage brought on by diabetes.

The 'marker' of enzyme activity in the tissues is frequently employed to detect the early harmful effects of foreign substances given to experimental animals. This is due to the fact that any changes in the metabolic processes caused by the presence of a xenobiotic in experimental animals will manifest as an increase or decrease in the activity of these enzymes. The marker enzymes AST, ALT, and ALP are signs of liver damage or a decline in liver function. They have significant metabolic effects. While ALT and AST are cytosolic enzymes, ALP is a membrane-bound enzyme. Oxaloacetate and glutamate are produced via the reductive transfer of an amino group from aspartate to -ketoglutarate, which is catalyzed by the AST. In gluconeogenesis and amino acid metabolism, ALT is crucial. The reductive transfer of an amino group from alanine to -ketoglutarate, which results in glutamate and pyruvate, is catalyzed. These marker enzymes are secreted from damaged tissues in a liver affected by hepatocellular lesions, and blood flow increases their levels (Suryawanshi *et al*., 2014 & Subbiah *et al*., 2018).

Two well-known diagnostic signs of liver disease are elevated ALT and AST levels. To distinguish liver injury from damage to other organs, one uses the ratio of these two enzymes in the serum. In reality, the biomarker of hepatotoxicity that is most frequently used is ALT activity. Because the body cannot produce these enzymes through bile as a result of liver congestion or biliary tract obstruction, hepatotoxicity causes these enzymes to be elevated above their usual levels. Typically, liver injury is accompanied by increased serum activity of this enzyme. Administration of the ethanol extract of *Justicia carnea* at 100mg/kg,200 mg/kg and 500mg/kg significantly ($P<0.05$) decreased the normal AST and ALT values and increased the ALP value (Nwodo *et al*., 2015).

These effects by the extracts are comparable with that of methformin (the standard drug) and the normal control group (1).The liver was damaged in this study as a result of diabetes. As seen in the untreated diabetic control group (2), these damages led to an increase in serum transaminases in which the levels of AST and ALT increased significantly, but ALP levels decreased. When *Justicia carnea's* methanol extract was given at doses of 100 mg/kg, 200 mg/kg and 500 mg/kg, the disturbed AST and ALT values were considerably ($P<0.05$) lowered, while the ALP value was significantly ($P<0.05$) elevated. These effects of the extracts are equivalent to those of the conventional medication methformin and the healthy control group (1).These readings being reduced by the *Justicia carnea* ethanol extract may indicate that the leaves of this plant have hepatoprotective properties. According to Singh *et al*., (2012) this activity would show up as stability of the hepatic membrane and regeneration of the hepatocytes. By reducing the tissue lesions, the extract may also have inhibited the release of hepatic enzymes at the bloodstream level. According to Sharma and

Shukla , the rise in ALP value after extract administration compared to the diabetic control group (2) receiving no treatment could have been caused by problems with secretary activity, metabolite transport, or other hepatotoxic circumstances. Furthermore, the maximal rise shown at the highest dose level of 500 mg/kg provided may potentially have been caused by the drug, as has been the case with overdoses of paracetamol, methyldopa, isonocizid, and certain steroids (Subbiah *et al*., 2018). In order to diagnose hepatobilliary disease or cholesterol blockage, alkaline phospatase is helpful.

When compared to the normal group(1), the untreated diabetic control group(2) showed a lower amount of total protein. When compared to the untreated diabetic control group(2), the administration of the extract to group 4,5 and 6 led to an increase in total protein, which suggests stabilization of the plasma membrane and revitalization of the liver cell membrane which may have been formerly damaged by diabetes. Additionally, it means that the liver's capacity for maintaining nutritional homeostasis and its synthetic activity was improved when the extract of *Justicia carnea* was administered.

The result of the weight of the animals gotten before inducing the diabetes (basal weight), 7days after treatment and after the 21days of treatment showed that diabetes has so much effect on the body weights of the rats. After 7days of induction of diabetes on the five groups(group 1-5), there was a 40% loss weight across the five groups when compared to the normal group (1). At the end of the 21days of treatment the *Justiscia carnea* extract treated group (group 4,5,6) and the methformin treated group(3) had a 20% weight recovering when compared to normal group (1), while the untreated diabetic group (2) had 50% weight loss when compared to normal(1).The weight gain by the extract treated diabetes rat suggests the reversal of diabetic effect of the streptozotocin by *Justicia carnea.*

From the result of phytochemical analysis, J*usticia carnea* is found to contain bioactive substance such as phenol and presence of antioxidant phytochemicals like flavonods, polyphenols, and tannins which acts as a free radical scavengers. Polyphenols are known to interact with proteins and can inhbit enzyme activity. The polyphenols have been reported to inhibit alpha amylase and alpha-glucosidase associated with lipid peroxidation and diabetes.

5.2 Conclusion

From this research, it can be concluded that *Justicia carnea* methanol leaf extract have the potential to reduce blood sugar level and can be used in the effective management of diabetes. This can be traced to its ameliorative activity on the damaged beta cells of the pancreatic cells caused by Streptozotocin. It also has the ability to protect the vital organs such as pancreas, liver and kidney from the complications of diabetes mellitus, by restoring to normalcy the functioning and production of biochemical markers such as enzymes and certain metabolites which serve as indications to organ failure which can be caused by complications due to diabetes mellitus. The result also indicates that, in addition to its hypoglycemic effects, *Justicia carnea* will be helpful in lowering the problems and metabolic syndrome that are frequently associate with diabetes.

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APPENDIX

CREATINE

Descriptives

VAR00002

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1	3	.2100	.04359	.02517	.1017	.3183	.16	.24
2	3	1.1800	.02000	.01155	1.1303	1.2297	1.16	1.20
3	4	.1450	.06191	.03096	.0465	.2435	.09	.23
4	4	.2175	.12842	.06421	.0132	.4218	.11	.40
5	3	.1933	.04041	.02333	.0929	.2937	.15	.23
6	4	.4925	.06752	.03376	.3851	.5999	.43	.58
Total	21	.3890	.35841	.07821	.2259	.5522	.09	1.20

ANOVA

VAR00002

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2.487	5	.497	90.406	.000
Within Groups	.083	15	.006		
Total	2.569	20			

Multiple Comparisons

VAR00002

LSD

(I) VAR00001	(J) VAR00001	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1	2	-.97000*	.06056	.000	-1.0991	-.8409
	3	.06500	.05665	.269	-.0557	.1857
	4	-.00750	.05665	.896	-.1282	.1132
	5	.01667	.06056	.787	-.1124	.1457
	6	-.28250*	.05665	.000	-.4032	-.1618
2	1	.97000*	.06056	.000	.8409	1.0991
	3	1.03500*	.05665	.000	.9143	1.1557
	4	.96250*	.05665	.000	.8418	1.0832

	5	.98667*	.06056	.000	.8576	1.1157
	6	.68750*	.05665	.000	.5668	.8082
3	1	-.06500	.05665	.269	-.1857	.0557
	2	-1.03500*	.05665	.000	-1.1557	-.9143
	4	-.07250	.05245	.187	-.1843	.0393
	5	-.04833	.05665	.407	-.1691	.0724
	6	-.34750*	.05245	.000	-.4593	-.2357
4	1	.00750	.05665	.896	-.1132	.1282
	2	-.96250*	.05665	.000	-1.0832	-.8418
	3	.07250	.05245	.187	-.0393	.1843
	5	.02417	.05665	.676	-.0966	.1449
	6	-.27500*	.05245	.000	-.3868	-.1632
5	1	-.01667	.06056	.787	-.1457	.1124
	2	-.98667*	.06056	.000	-1.1157	-.8576
	3	.04833	.05665	.407	-.0724	.1691
	4	-.02417	.05665	.676	-.1449	.0966
	6	-.29917*	.05665	.000	-.4199	-.1784
6	1	.28250*	.05665	.000	.1618	.4032
	2	-.68750*	.05665	.000	-.8082	-.5668
	3	.34750*	.05245	.000	.2357	.4593
	4	.27500*	.05245	.000	.1632	.3868
	5	.29917*	.05665	.000	.1784	.4199

*.The mean difference is significant at the 0.05 level.

TOTAL PROTEIN

Descriptives

VAR00001

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	3.9600	1.17690	.67949	1.0364	6.8836	3.15	5.31
2.00	3	6.2600	.87504	.50521	4.0863	8.4337	5.53	7.23
3.00	3	6.4600	.26153	.15100	5.8103	7.1097	6.16	6.64
4.00	3	5.8300	.26889	.15524	5.1620	6.4980	5.66	6.14
5.00	3	5.8267	.59164	.34158	4.3570	7.2964	5.39	6.50
6.00	3	5.4033	.28006	.16169	4.7076	6.0990	5.12	5.68
Total	18	5.6233	1.01325	.23882	5.1195	6.1272	3.15	7.23

ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.013	5	2.403	5.300	.008
Within Groups	5.440	12	.453		
Total	17.453	17			

Multiple Comparisons

Dependent Variable: VAR00001

	(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1.00	2.00	-2.30000*	.54974	.001	-3.4978	-1.1022
		3.00	-2.50000*	.54974	.001	-3.6978	-1.3022
		4.00	-1.87000*	.54974	.005	-3.0678	-.6722
		5.00	-1.86667*	.54974	.005	-3.0645	-.6689
		6.00	-1.44333*	.54974	.022	-2.6411	-.2455
	2.00	1.00	2.30000*	.54974	.001	1.1022	3.4978
		3.00	-.20000	.54974	.722	-1.3978	.9978
		4.00	.43000	.54974	.449	-.7678	1.6278

	5.00	.43333	.54974	.446	-.7645	1.6311
	6.00	.85667	.54974	.145	-.3411	2.0545
	1.00	2.50000*	.54974	.001	1.3022	3.6978
	2.00	.20000	.54974	.722	-.9978	1.3978
3.00	4.00	.63000	.54974	.274	-.5678	1.8278
	5.00	.63333	.54974	.272	-.5645	1.8311
	6.00	1.05667	.54974	.079	-.1411	2.2545
	1.00	1.87000*	.54974	.005	.6722	3.0678
	2.00	-.43000	.54974	.449	-1.6278	.7678
4.00	3.00	-.63000	.54974	.274	-1.8278	.5678
	5.00	.00333	.54974	.995	-1.1945	1.2011
	6.00	.42667	.54974	.453	-.7711	1.6245
	1.00	1.86667*	.54974	.005	.6689	3.0645
	2.00	-.43333	.54974	.446	-1.6311	.7645
5.00	3.00	-.63333	83 74	.272	-1.8311	.5645
	4.00	-.00333	.54974	.995	-1.2011	1.1945
	6.00	.42333	.54974	.456	-.7745	1.6211
	1.00	1.44333*	.54974	.022	.2455	2.6411
	2.00	-.85667	.54974	.145	-2.0545	.3411
6.00	3.00	-1.05667	.54974	.079	-2.2545	.1411
	4.00	-.42667	.54974	.453	-1.6245	.7711
	5.00	-.42333	.54974	.456	-1.6211	.7745

*. The mean difference is significant at the 0.05 level.

TRIGS

Descriptives

VAR00001

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	2.8688	.03412	.01970	2.7840	2.9535	2.83	2.89
2.00	3	2.5834	.26995	.15586	1.9128	3.2540	2.36	2.88
3.00	3	2.3731	.02753	.01590	2.3047	2.4415	2.34	2.40
4.00	3	2.3191	.02268	.01309	2.2627	2.3754	2.30	2.34
5.00	3	2.3491	.04963	.02866	2.2258	2.4724	2.30	2.40
6.00	3	2.3010	.02081	.01202	2.2493	2.3527	2.29	2.33
Total	18	2.4657	.22980	.05417	2.3515	2.5800	2.29	2.89

ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.741	5	.148	11.375	.000
Within Groups	.156	12	.013		
Total	.898	17			

Multiple Comparisons

Dependent Variable: VAR00001

	(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1.00	2.00	.28538*	.09322	.010	.0823	.4885
		3.00	.49565*	.09322	.000	.2925	.6988
		4.00	.54972*	.09322	.000	.3466	.7528
		5.00	.51968*	.09322	.000	.3166	.7228
		6.00	.56775*	.09322	.000	.3646	.7709
	2.00	1.00	-.28538*	.09322	.010	-.4885	-.0823
		3.00	.21028*	.09322	.044	.0072	.4134
		4.00	.26435*	.09322	.015	.0612	.4675
		5.00	.23431*	.09322	.027	.0312	.4374
		6.00	.28237*	.09322	.010	.0793	.4855
	3.00	1.00	-.49565*	.09322	.000	-.6988	-.2925
		2.00	-.21028*	.09322	.044	-.4134	-.0072
		4.00	.05407	.09322	.573	-.1490	.2572
		5.00	.02403	.09322	.801	-.1791	.2271
		6.00	.07209	.09322	.454	-.1310	.2752
	4.00	1.00	-.54972*	.09322	.000	-.7528	-.3466
		2.00	-.26435*	.09322	.015	-.4675	-.0612
		3.00	-.05407	.09322	.573	-.2572	.1490

	5.00	-.03004	.09322	.753	-.2331	.1731
	6.00	.01802	.09322	.850	-.1851	.2211
	1.00	-.51968*	.09322	.000	-.7228	-.3166
	2.00	-.23431*	.09322	.027	-.4374	-.0312
5.00	3.00	-.02403	.09322	.801	-.2271	.1791
	4.00	.03004	.09322	.753	-.1731	.2331
	6.00	.04806	.09322	.616	-.1550	.2512
	1.00	-.56775*	.09322	.000	-.7709	-.3646
	2.00	-.28237*	.09322	.010	-.4855	-.0793
6.00	3.00	-.07209	.09322	.454	-.2752	.1310
	4.00	-.01802	.09322	.850	-.2211	.1851
	5.00	-.04806	.09322	.616	-.2512	.1550

*. The mean difference is significant at the 0.05 level.

WBC

Descriptives

VAR00001

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	17.2667	5.26245	3.03827	4.1940	30.3393	11.20	20.60
2.00	3	9.9333	3.93234	2.27034	.1648	19.7018	5.50	13.00
3.00	3	17.6000	.00000	.00000	17.6000	17.6000	17.60	17.60
4.00	3	8.6000	4.60000	2.65581	-2.8270	20.0270	4.00	13.20
5.00	3	15.6000	3.37787	1.95021	7.2089	23.9911	11.70	17.60
6.00	3	16.3000	3.50000	2.02073	7.6055	24.9945	12.80	19.80
Total	18	14.2167	4.89084	1.15278	11.7845	16.6488	4.00	20.60

ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	230.692	5	46.138	3.147	.048
Within Groups	175.953	12	14.663		
Total	406.645	17			

Multiple Comparisons

Dependent Variable: VAR00001

	(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1.00	2.00	7.33333*	3.12653	.037	.5212	14.1455

	3.00	-.33333	3.12653	.917	-7.1455	6.4788
	4.00	8.66667*	3.12653	.017	1.8545	15.4788
	5.00	1.66667	3.12653	.604	-5.1455	8.4788
	6.00	.96667	3.12653	.762	-5.8455	7.7788
	1.00	-7.33333*	3.12653	.037	-14.1455	-.5212
	3.00	-7.66667*	3.12653	.030	-14.4788	-.8545
2.00	4.00	1.33333	3.12653	.677	-5.4788	8.1455
	5.00	-5.66667	3.12653	.095	-12.4788	1.1455
	6.00	-6.36667	3.12653	.064	-13.1788	.4455
	1.00	.33333	3.12653	.917	-6.4788	7.1455
	2.00	7.66667*	3.12653	.030	.8545	14.4788
3.00	4.00	9.00000*	3.12653	.014	2.1879	15.8121
	5.00	2.00000	3.12653	.534	-4.8121	8.8121
	6.00	1.30000	3.12653	.685	-5.5121	8.1121
	1.00	-8.66667*	3.12653	.017	-15.4788	-1.8545
	2.00	-1.33333	3.12653	.677	-8.1455	5.4788
4.00	3.00	-9.00000*	3.12653	.014	-15.8121	-2.1879
	5.00	-7.00000*	3.12653	.045	-13.8121	-.1879
	6.00	-7.70000*	3.12653	.030	-14.5121	-.8879
	1.00	-1.66667	3.12653	.604	-8.4788	5.1455
	2.00	5.66667	3.12653	.095	-1.1455	12.4788
5.00	3.00	-2.00000	3.12653	.534	-8.8121	4.8121
	4.00	7.00000*	3.12653	.045	.1879	13.8121
	6.00	-.70000	3.12653	.827	-7.5121	6.1121
6.00	1.00	-.96667	3.12653	.762	-7.7788	5.8455

	2.00	**6.36667**	**3.12653**	**.064**	**-.4455**	**13.1788**
	3.00	**-1.30000**	**3.12653**	**.685**	**-8.1121**	**5.5121**
	4.00	**7.70000***	**3.12653**	**.030**	**.8879**	**14.5121**
	5.00	**.70000**	**3.12653**	**.827**	**-6.1121**	**7.5121**

***. The mean difference is significant at the 0.05 level.**

HDL

Descriptives

VAR00001

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	187.5778	49.81736	28.76207	63.8246	311.3310	152.92	244.67
2.00	3	106.0222	61.26853	35.37340	-46.1772	258.2217	36.70	152.92
3.00	3	83.5944	23.15733	13.36989	26.0685	141.1204	67.28	110.10
4.00	3	212.0444	25.46571	14.70264	148.7841	275.3048	183.50	232.43
5.00	3	89.7111	40.72677	23.51361	-11.4598	190.8820	55.05	134.57
6.00	3	75.4389	12.73286	7.35132	43.8087	107.0691	61.17	85.63
Total	18	125.7315	64.29683	15.15491	93.7574	157.7055	36.70	244.67

ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	51797.066	5	10359.413	6.726	.003
Within Groups	18482.324	12	1540.194		
Total	70279.390	17			

Multiple Comparisons

Dependent Variable: VAR00001

	(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1.00	2.00	81.55556*	32.04365	.026	11.7384	151.3727
		3.00	103.98333*	32.04365	.007	34.1662	173.8005
		4.00	-24.46667	32.04365	.460	-94.2838	45.3505
		5.00	97.86667*	32.04365	.010	28.0495	167.6838
		6.00	112.13889*	32.04365	.004	42.3218	181.9560
	2.00	1.00	-81.55556*	32.04365	.026	-151.3727	-11.7384
		3.00	22.42778	32.04365	.497	-47.3893	92.2449
		4.00	-106.02222*	32.04365	.006	-175.8393	-36.2051
		5.00	91 L	32.04365	.620	-53.5060	86.1282
		6.00	30.58333	32.04365	.359	-39.2338	100.4005
	3.00	1.00	-103.98333*	32.04365	.007	-173.8005	-34.1662
		2.00	-22.42778	32.04365	.497	-92.2449	47.3893
		4.00	-128.45000*	32.04365	.002	-198.2671	-58.6329
		5.00	-6.11667	32.04365	.852	-75.9338	63.7005
		6.00	8.15556	32.04365	.803	-61.6616	77.9727
	4.00	1.00	24.46667	32.04365	.460	-45.3505	94.2838
		2.00	106.02222*	32.04365	.006	36.2051	175.8393
		3.00	128.45000*	32.04365	.002	58.6329	198.2671
		5.00	122.33333*	32.04365	.002	52.5162	192.1505
		6.00	136.60556*	32.04365	.001	66.7884	206.4227
	5.00	1.00	-97.86667*	32.04365	.010	-167.6838	-28.0495
		2.00	-16.31111	32.04365	.620	-86.1282	53.5060
		3.00	6.11667	32.04365	.852	-63.7005	75.9338
		4.00	-122.33333*	32.04365	.002	-192.1505	-52.5162
		6.00	14.27222	32.04365	.664	-55.5449	84.0893
	6.00	1.00	-112.13889*	32.04365	.004	-181.9560	-42.3218
		2.00	-30.58333	32.04365	.359	-100.4005	39.2338
		3.00	-8.15556	32.04365	.803	-77.9727	61.6616
		4.00	-136.60556*	32.04365	.001	-206.4227	-66.7884
		5.00	-14.27222	32.04365	.664	-84.0893	55.5449

*. The mean difference is significant at the 0.05 level.

ALP

Descriptives

VAR00001

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	.0347	.00929	.00536	.0116	.0577	.03	.05
2.00	3	.1677	.04423	.02554	.0578	.2775	.14	.22
3.00	3	.1213	.12208	.07048	-.1819	.4246	.04	.26
4.00	3	.2187	.09777	.05645	-.0242	.4615	.12	.31
5.00	3	.2143	.07047	.04068	.0393	.3894	.16	.29
6.00	3	.0537	.01159	.00669	.0249	.0825	.05	.07
Total	18	.1351	.09607	.02264	.0873	.1828	.03	.31

ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.094	5	.019	3.557	.033
Within Groups	.063	12	.005		
Total	.157	17			

Multiple Comparisons

Dependent Variable: VAR00001

	(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1.00	2.00	-.13300*	.05926	.044	-.2621	-.0039
		3.00	-.08667	.05926	.169	-.2158	.0424
		4.00	-.18400*	.05926	.009	-.3131	-.0549
		5.00	-.17967*	.05926	.010	-.3088	-.0506
		6.00	-.01900	.05926	.754	-.1481	.1101
	2.00	1.00	.13300*	.05926	.044	.0039	.2621
		3.00	.04633	.05926	.449	-.0828	.1754
		4.00	-.05100	.05926	.406	-.1801	.0781
		5.00	-.04667	.05926	.446	-.1758	.0824

	6.00	.11400	.05926	.078	-.0151	.2431
	1.00	.08667	.05926	.169	-.0424	.2158
	2.00	-.04633	.05926	.449	-.1754	.0828
3.00	4.00	-.09733	.05926	.126	-.2264	.0318
	5.00	-.09300	.05926	.143	-.2221	.0361
	6.00	.06767	.05926	.276	-.0614	.1968
	1.00	.18400*	.05926	.009	.0549	.3131
	2.00	.05100	.05926	.406	-.0781	.1801
4.00	3.00	.09733	.05926	.126	-.0318	.2264
	5.00	.00433	.05926	.943	-.1248	.1334
	6.00	.16500*	.05926	.017	.0359	.2941
	1.00	.17967*	.05926	.010	.0506	.3088
	2.00	.04667	.05926	.446	-.0824	.1758
5.00	3.00	.09300	.05926	.143	-.0361	.2221
	4.00	-.00433	.05926	.943	-.1334	.1248
	6.00	.16067*	.05926	.019	.0316	.2898
	1.00	.01900	.05926	.754	-.1101	.1481
	2.00	-.11400	.05926	.078	-.2431	.0151
6.00	3.00	-.06767	.05926	.276	-.1968	.0614
	4.00	-.16500*	.05926	.017	-.2941	-.0359
	5.00	-.16067*	.05926	.019	-.2898	-.0316

*. The mean difference is significant at the 0.05 level.

LYM

Descriptives

VAR00001

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
1.00	3	10.6000	1.99249	1.15036	5.6504	15.5496	9.40	12.90
2.00	3	8.5667	3.42685	1.97850	.0539	17.0794	4.80	11.50
3.00	3	15.3000	.00000	.00000	15.3000	15.3000	15.30	15.30
4.00	3	7.5000	4.20000	2.42487	-2.9334	17.9334	3.30	11.70
5.00	3	11.4000	4.32088	2.49466	.6663	22.1337	6.70	15.20
6.00	3	12.0000	1.80000	1.03923	7.5286	16.4714	10.20	13.80
Total	18	10.8944	3.63212	.85610	9.0882	12.7007	3.30	15.30

ANOVA

VAR00001

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	113.743	5	22.749	2.470	.093
Within Groups	110.527	12	9.211		
Total	224.269	17			

Multiple Comparisons

Dependent Variable: VAR00001

	(I) VAR00002	(J) VAR00002	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	1.00	2.00	2.03333	2.47798	.428	-3.3657	7.4324
		3.00	-4.70000	2.47798	.082	-10.0990	.6990
		4.00	3.10000	2.47798	.235	-2.2990	8.4990
		5.00	-.80000	2.47798	.752	-6.1990	4.5990
		6.00	-1.40000	2.47798	.582	-6.7990	3.9990
	2.00	1.00	-2.03333	2.47798	.428	-7.4324	3.3657
		3.00	-6.73333*	2.47798	.019	-12.1324	-1.3343
		4.00	1.06667	2.47798	.674	-4.3324	6.4657
		5.00	-2.83333	2.47798	.275	-8.2324	2.5657
		6.00	-3.43333	2.47798	.191	-8.8324	1.9657

	1.00	4.70000	2.47798	.082	-.6990	10.0990
	2.00	6.73333*	2.47798	.019	1.3343	12.1324
3.00	4.00	7.80000*	2.47798	.008	2.4010	13.1990
	5.00	3.90000	2.47798	.142	-1.4990	9.2990
	6.00	3.30000	2.47798	.208	-2.0990	8.6990
	1.00	-3.10000	2.47798	.235	-8.4990	2.2990
	2.00	-1.06667	2.47798	.674	-6.4657	4.3324
4.00	3.00	-7.80000*	2.47798	.008	-13.1990	-2.4010
	5.00	-3.90000	2.47798	.142	-9.2990	1.4990
	6.00	-4.50000	2.47798	.094	-9.8990	.8990
	1.00	.80000	2.47798	.752	-4.5990	6.1990
	2.00	2.83333	2.47798	.275	-2.5657	8.2324
5.00	3.00	-3.90000	2.47798	.142	-9.2990	1.4990
	4.00	3.90000	2.47798	.142	-1.4990	9.2990
	6.00	-.60000	2.47798	.813	-5.9990	4.7990
	1.00	1.40000	2.47798	.582	-3.9990	6.7990
	2.00	3.43333	2.47798	.191	-1.9657	8.8324
6.00	3.00	-3.30000	2.47798	.208	S-8.6990	2.0990
	4.00	4.50000	2.47798	.094	-.8990	9.8990
	5.00	.60000	2.47798	.813	-4.7990	5.9990

*. The mean difference is significant at the 0.05 level.