RENOPROTECTIVE POTENTIAL OF DCM FRACTION OF GL STEM BARK IN STREPTOZOTOCIN INDUCED DIABETES IN WISTAR RATS

BY

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CERTIFICATION

We, the undersigned do certify that this research was carried out by **IGBONAJU IFUNANYA SANDRA** with (**MAT NO**. **LSC1605089**) in the Department of Biochemistry. This work is adequate in scope and qualifies for the partial fulfillment for the award of BACHELOR OF SCIENCE of the University of Benin.

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I hereby declare that this work is based on personal research undertaken by me in the Department of Biochemistry, Faculty of Life Sciences, University of Benin under the supervision of DR.S.I OJEABURU

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DEDICATION

This work is dedicated to God Almighty for the grace to see this project through to the very end.

ACKNOWLEDGEMENT

I wish to register my profound gratitude to God Almighty for his grace, mercies and assistance all through my life.

Special thanks to the Head of Department, PROF. (MRS) K. IMAFIDON for her support, moral guidance and academic advice all through these years.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Persistent hyperglycemia is a symptom of diabetes mellitus, a metabolic or hormonal disorder caused by problems with insulin secretion. Type 1 (T1DM) and type 2 (T2DM) diabetes mellitus are the two kinds of the disease. T1DM is also known as insulin-dependent diabetes mellitus (IDDM), and it is brought on by a problem with insulin synthesis. T2DM, however, is often referred to as non-insulin dependent diabetes mellitus since it is linked to cells' inability to respond to insulin (insulin resistance) (NIDDM). All across the world, diabetes is becoming more common. According to estimates, 415 million adults had diabetes in 2015, and by 2040, 642 million persons were expected to have the disease (Rahelic, 2016). More than 70% of people with T2DM reside in developing nations, and this number is rising yearly. There are several pharmacological antidiabetic medicines on the market, but the rise in the prevalence of T2DM, particularly in poor nations, together with the side effects linked to these drugs, has brought attention to the need for more efficient, safer, and affordable management techniques. Over the past ten years, there has been a sharp rise in the use of complementary and alternative medicine (CAM) worldwide for the treatment of conditions including diabetes. According to reports, up to 72.8% of diabetics took herbal remedies. food supplements and other CAM treatments (Chang et al., 2007). Additionally, data shows that the majority of patients who use CAM therapies do so in addition to traditional therapy rather than in place of it (Kiran et al., 2012). There are many medicinal plants that are thought to have antidiabetic effects and have been used to treat diabetes (Ghorbani, 2014). Due to one or more of their phytochemical qualities, many of the plants found in Nigeria are known for their medicinal properties. (Okwu, 2003). These plants frequently display a variety of

biological and pharmaceutical qualities, including anti-inflammatory, anti-bacterial, anti-fungal, etc (Ekeke et al, 2003). Despite the availability of contemporary medicine formulations, many ailments are treated with extracts from plant roots and bark (Sarkar, 2015). The mainstay of medical care has always been and will continue to be natural products.

JUSTIFICATION OF STUDY

The present study is to validate the claim that DCM fraction of *Gongronema latifolium* extract has antidiabetic properties to control blood glucose level. The detection, prevention, treatment and control of this burden must be a top priority. Due to high cost of therapeutic drugs, many patients turn to traditional herbal medicine for the management of diabetes. Thus scientific validation and rationalization of medicinal plants need to be intensified. Indeed, diverse pharmacological benefits have been attributed to *Gongronema latifolium* extract as herbal medicine due to the high level of phytochemicals and antioxidants. Antioxidants are molecules that have the ability to scavenge free radicals produced as a result of reactive oxygen species which causes the development of diabetes. Certainly, this validation will serve as criteria to recommend for the continuous use of *Gongronema latifolium* extract in ethno medicine.

1.2 AIM OF STUDY

The aim of this study is to determine the effects of DCM fraction of *Gongronema latifolium* extract on STZ induced diabetic rats in comparison with a standard insulin drug commonly used for diabetic patients.

1.3 OBJECTIVES OF STUDY

The following are the objectives of this study are:

1. To determine it's ameliorating potential to manage diabetic related complication.

2. To evaluate the effects of DCM fraction of Gongronema latifolium extract on diabetes induced rats in relation to:

3. kidney function

1.4 LITERATURE REVIEW

1.4.1 MEDICINAL PLANTS

Ten percent of all vascular plants are used as medicinal plants (Fonnegra, 2007), and there are thought to be between 350,000 and nearly half a million species of them. Plants have been well documented for their medicinal uses for thousands of years, and traditional medicines are still a major part of habitual treatments of various maladies in different parts of the world. In Africa and other impoverished nations, medicinal plants offer a rich source of raw ingredients for primary healthcare. One of the primary sources of biologically active materials is thought to be plants. Finding new medicines has benefited greatly from the phytochemical screening of medicinal plants. Many therapeutic plants have been the subject of thorough chemical analyses, which have resulted in the isolation of pure bioactive compounds that have undergone pharmacological testing. Novel medications have therefore been found, along with new applications (Pimm et al., 2014). Secondary metabolites of various kinds are present in medicinal plants and are important in the pathogenesis of numerous diseases. They are also used in the production of pharmaceuticals. The bioactive components or plant extracts may be employed as a new formulation for the revolutionary drug to treat a variety of ailments. discovering new medications for the pharmaceutical businesses (Singh et al., 2017). The chemical active compounds in plants that create specific physiological effects on the human body are what give them their therapeutic worth (Yadav

et al., 2017). Plants are thought to be a rich source of bioactive compounds and could serve as a substitute for other sources of insect repellents (Narendiran et al., 2016). Plant-derived secondary metabolites, often known as phytochemicals, have notable pharmacological properties, including anti-oxidative, anti-allergic, antibacterial, hypoglycemic, and anti-carcinogenic properties. The cells are shielded by these secondary metabolites from the harm that unstable molecules known as free radicals can do (Harini and Nithyalakshmi, 2017). Utilizing natural antibacterial substances, particularly those derived from plants, for food preservation is gaining popularity. the requirement to look for medicinal herbs (Chavan, 2016). However, older men and women hold the expertise and awareness of herbal medicines. women who fall between the ages of 41 and 70. 2017 (Sharma). The objectives of using plants as sources of therapeutic agents, according to Fabricant and Farnsworth (2001), are to isolate bioactive compounds for direct use as drugs, produce bioactive compounds of novel or known structures as lead compounds for semi-synthesis, produce patentable entities of higher activity and/or lower toxicity, use agents as pharmacologic tools, and use the whole plant or part of it as a herbal remedy. The isolation of molecules from plants and other natural sources, synthetic chemistry, combinatorial chemistry, and molecular modeling are only a few of the techniques used in drug development (Lombardino, 2004).

Drugs generated from medicinal plants can function as both new drugs and leads for pharmaceutical and synthetic chemists to optimize (Balunas, 2005). Traditional medicine is a term that refers to the progressive refinement of the use of these plants through many generations.

1.4.2 TRADITIONAL MEDICINE

Traditional medicine refers to the theories, beliefs, and practices that are inherent to various cultures, whether or not they can be rationalized, and that are used to maintain health as well as to prevent, diagnose, treat, or improve physical and mental illness (WHO, 2000). Minerals, animal parts, and herbal medicines are all employed in its practice; however, herbal medicines are the most frequently used of the three, hence they will be focused on in this study. People have utilized plants as medicine for ages. Throughout history, plant-based foods or botanical parts and powder have been employed, with varied degrees of success, to treat and prevent ailments (Maryam, 2010). The last ten years have seen a global expansion and popularity of traditional medicine (WHO, 2000). Traditional medical practices differ widely from nation to nation and area to region due to influences from culture, history, philosophy, and individual attitudes. Their theory and practice frequently diverge significantly from those of mainstream medicine (WHO, 2000). The inheriting person is typically shown the plant, given the common name, and instructions on how to create the drug from the plant or plant part for a particular disease in accordance with tradition (John, 1995). Through trial and error, the historical usage of traditional medicine that has been passed down from generation to generation has shown some degree of safety and efficacy. But more scientific investigation is required to show its safety and effectiveness (WHO, 2000). The widespread use of traditional medicinal plants in developing nations is sometimes linked to their availability and affordability as a primary healthcare resource, particularly for the world's poorest people. According to estimates from the World Health Organization (WHO), around 80% of Africans use traditional medicine, and about 85% of Africans also use plant extracts (Farnsworth. 2001). Traditional healers are consulted by 20% of patients who seek conventional medical care, making them a substantial source of healthcare for many Africans (kareru, 2007). There are a lot of medicine plants utilized overall, including both native and foreign species (Maryam, 2010). It is expected that in order to understand the

magnitude of this relationship that there is one traditional healer for every 50,000 people in Sub-Saharan Africa, but only one doctor for every 40,000 people (Sandiga, 1995). Additionally, the distribution of such persons may be uneven, with the majority being located in cities or other urban regions and being inaccessible to rural residents. For the treatment of microbiological illnesses, herbal remedies are far less expensive, and frequently, they are paid for in kind or in accordance with the "wealth" of the customer. Scientific proof of the effectiveness and safety of traditional medicine claims, however, has not expanded along with the usage of traditional medicinal plants (WHO, 2000). Traditional medicine is also highly popular in many developing countries because it is firmly embedded within wider belief systems and yet little effort has been devoted to their documentation, validation, utilization and conservation. With respect to diseases caused by microorganisms, the increasing resistance in many common pathogens to currently used therapeutic agents, such as antibiotics and antiviral agents, has led to renewed interest in the discovery of novel anti-effective compounds (Maryam, 2010). Although it's possible that traditional herbalists are unaware of the precise bioactive substances found in the medicines they utilize. The key to traditional herbal medicine's success in treating a variety of human illnesses is the presence of bioactive chemicals (kareru, 2007).

1.4.3 DESCRIPTION

G. latifolium (utazi) is a climbing shrub with broad, heart-shaped leaves that has a characteristic sharp, bitter and slightly sweet taste, especially when eaten fresh. The stems have soft/hairy that yields milky latex or exudates(Juarez-Rojop et al., 2012).

Fig., 4: Leaves of *G. latifolium*

Source: Ojo *et al*., 2014

Gongronema latifolium

Gongronema latifolium In numerous tropical African cultures, the herb Gongronema latifolium is used for both nutritional and ethnomedical purposes.

Benth's *Gongronema latifolium* is a member of the Asclepiadaceae family. It is a plant that is both edible and medicinal, and it is primarily found in Nigeria and other tropical African nations' rain forests. The plant can be multiplied through stem cuttings or seeds, and it has yellow blooms and white latex. The Ikales of Nigeria's Ondo State refer to G. latifolium as Iteji. The plant is known by the names Utazi (Ibo), Utasi (Efik/Ibibio), and Arokeke (Yoruba). In this review, many authors' scientific reports on the chemical makeup and bioactivity (anti-inflammatory, antibacterial, antidiabetic, antioxidant, anticancer, and allelopathic capabilities) of plant material are discussed. Future prospects for plant extracts in the fields of alcoholic fermentation, beer, herbal remedies, and food preservation, drug discovery and allelopathy are also highlighted.

Gongronema latifolium, commonly called ‘utazi’ and ‘arokeke’ in the South Western and South Eastern parts of Nigeria, is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine (Ugochukwu and Babady, 2002; Ugochukwu et al., 2013).

1.5 PHYTOCHEMICAL AND NUTRITIONAL COMPOSITION

Iwu reported flavones and sterols as active constituents of *G. latifolium*. Morebise and Fafunso reported the presence of saponins and flavonoids in the methanolic extract of G. latifolium leaves. Eze and Nwanguma reported the occurrence of tannins in the leaves of the plant. B-Sitosterol, lupenyl esters, pregnane ester and essential oils have also been reported to be present in the body parts . Saponins are bioactive glycosides of steroids or triterpenes . The triterpene or steroidal rings are usually referred to as sapogenins or aglycones . The saponins interact with cholesterol, bile acids, and other 3-hydroxy steroids to create mixed micelles, have a bitter taste, produce stable foams in aqueous solutions, and have moderate cytotoxicity. Numerous other medicinal plants are also known to be abundant in saponins, in addition to *Gongronema latifolium*. Examples include Vernonia amygdalina, Liquorice, Buplekum falcatum, and Panax ginseng. Flavonoids are extensively spread and make up the majority of the plant's coloring. They are a sizable group of phenolic chemicals that are involved in numerous pharmacological processes. They can be further classified into other classes such the flavones, flavonols, and flavanones and are found as aglycones, glycosides, and methylated derivatives. It has been demonstrated that several plant flavonoids have anti-infectious, anti-cardiovascular, anti-cancer, and anti-aging properties.

1.5.1 ANTIDIABETIC ACTIVITY OF *GONGRONEMA LATIFOLIUM*

There have been numerous reports on the antidiabetic properties of *G. latifolium* leaf extracts. Experimental rats were given diabetes mellitus caused by streptozotocin in Sylvester *et al* study, .'s which was then treated with *G. latifolium* leaf extracts. According to their findings, the extracts considerably (P=0.05) reduced the diabetic rats' blood glucose levels by 66.34%. Additionally, they noted that as compared to the normal control, diabetes induction led to appreciable (P=0.05) increases in both total cholesterol (TC) and LDL cholesterol (54.42% and 55.0%, respectively) (NC). These were dramatically reduced by treatments with the extracts by 58.70% (TC) and 71.70% (LDL), respectively. When compared to the diabetic control group, the extract-treated mice had considerably reduced levels of the AST and ALT enzymes. Additionally, Edet et al. published their research on the effects of *G. latifolium* leaf extracts on allozan-induced diabetic rats. The serum activity of the following enzymes—creatine kinase (CK), CKMB isoform, lactate dehydrogenase, alanine aminotransferase (ALT), and aspartate aminotransferase (AST)—were found to be reduced by the extracts in their 2009 research when compared to controls. They came to the conclusion that G. latifolium extracts might protect the heart and skeletal muscles in cases of cardiac and skeletal muscle diseases. In their 2011 study, the researchers found that *G. latifolium* leaf extracts prevented male Wistar rats from losing weight and reversing the changes in haematological indices (WBC counts, hemoglobin counts, and packed cell volume) brought on by alloxan-induced diabetes. The antidiabetic and antiulcerogenic effects of *G. latifolium* leaf extracts on Streptozotocin-induced diabetic rats were also reported by Owu et al. They also claimed that the extract significantly brought the blood sugar levels of the diabetic animals down to levels comparable to the control animals who did not have diabetes.

1.5.2 REPORTED TOXICITY OF *GONGRONEMA LATIFOLIUM*

Sprague Dawley rats, both male and female, were used in this repeated dose 90-day oral toxicity research to assess the safety profile of the ethanolic extract of *G. latifolium* (GLES) leaves.

Mortality was not seen, nor were there any behavioral or physiological alterations. General hematological indicators were not significantly affected by GLES, and nephrotoxicity was not precipitated. By the end of the experiment, however, male rats given GLES3 had lower levels of serum triglycerides, total cholesterol, and low-density lipoprotein than the control group, and their corresponding retroperitoneal fat depots of white adipose tissue had been depleted. Both male and female GLES-treated rats had considerably enlarged livers. The highest dose resulted in detrimental gender-specific changes. Due to significant body weight gain and cerebrum weight loss, adverse risk was clearly visible in female rats.

1.5.3 TYPES OF DIABETES

The types of diabetes are:

Type 1 diabetes: This type is an autoimmune disease, meaning your body attacks itself. In this case, the insulin-producing cells in your pancreas are destroyed. Up to 10% of people who have diabetes have Type 1. It's usually diagnosed in children and young adults (but can develop at any age). It was once better known as "juvenile" diabetes. People with Type 1 diabetes need to take insulin every day. This is why it is also called insulin-dependent diabetes.

Gestational diabetes: This kind appears in some pregnant women. After pregnancy, gestational diabetes typically disappears. However, if you have gestational diabetes, you are more likely to eventually acquire Type 2 diabetes.

Less common types of diabetes include:

- Monogenic diabetes syndromes: Up to 4% of cases of diabetes are caused by these uncommon hereditary types of the disease. Examples include young-onset diabetes with maturity and neonatal diabetes.

- Diabetes associated with cystic fibrosis: This is a type of diabetes that only affects those who have this condition.

- Diabetes brought on by drugs or chemicals: Symptoms of this type include organ transplantation, HIV/AIDS therapy, and usage of glucocorticoids.

1.5.4 Classification of the diabetes mellitus

An proper classification is a key necessity for systematic epidemiologic and clinical research on and for the management of diabetes mellitus. The ability to recognize and discriminate between a disease's numerous forms and fit them into a logical etiopathologic framework is also a requirement for investigating a disease's natural history and genesis (Harris and Zimmet, 1997). Based on research on this diverse disease, the National Diabetes Data Group created the modern classification of diabetes and other types of glucose intolerance in 1979. Insulin-dependent diabetes mellitus (IDDM, type I diabetes), and non-insulin-dependent diabetes are the two main types of diabetes recognized in Western nations.

Dependent diabetes (NIDDM, type II diabetes). The evidence of this heterogeneity is overwhelming and includes the following:

There are many distinct disorders, most of which are individually rare, in which glucose intolerance is a feature;

Worldwide, there are significant disparities in the prevalence of the main types of diabetes among different racial or ethnic groups;

Clinical characteristics of glucose intolerance vary, for instance, the distinctions between obese, non-ketotic insulin resistant diabetes and thin, ketosis-prone insulin dependent diabetes;

Genetic, immunologic and clinical studies show that in Western countries, the forms of diabetes with their onset primarily in youth or in adulthood are distinct entities;e) the type of non-insulin requiring diabetes in young people, which is inherited in an autosomal dominant fashion is clearly different from the classic acute diabetes of juveniles; and in tropical countries, several clinical presentations occur, including fibrocalcific pancreatitis and malnutrition-related diabetes. This and other collective evidence have been used to divide diabetes mellitus into four distinct types namely;

- Insulin dependent diabetes,
- Non-insulin dependent diabetes,
- Malnutrition-related diabetes,

other types of diabetes.

The classification highlights the marked heterogeneity of the diabetic syndrome. Such heterogeneity has important implications not only for clinical management of diabetes but also for biomedical research (Harris and Zirnmet, 1997). In this study the focus was mainly on type II diabetes while type I diabetes was discussed briefly to point out the differences between the two types of diabetes.

Insulin dependent diabetes mellitus (IDDM)

Type I diabetes, which is a subclass of the disease, is typically distinguished by the sudden onset of severe symptoms, the need for exogenous insulin to maintain life, and a propensity for ketosis even in the basal state. All of these characteristics are brought on by absolute

insulin deficiency. Juvenile diabetes, also known as IDDM, is the most common type of diabetes in children and young adults in poor nations (Harris and Zimmet, 1997). It is a catabolic disorder in which circulating insulin is virtually absent, plasma glucagon is elevated, and the pancreatic B cells fail to respond to all insulinogenic stimuli (Nolte and Karam, 2001). People with type I diabetes are believed to have immune systems that are genetically prone to mount an aggressive autoimmune response against pancreatic B cell antigens as a result of a viral or toxic environmental event. Damage from viruses like the mumps virus and coxsackie virus B4, from chemical agents, or from damaging cytotoxins and antibodies generated from sensitized immunocytes are examples of extrinsic stimuli that could influence B cell activity.

A person may be predisposed to developing B cell failure after viral infections if they have an underlying genetic condition that affects pancreatic B cell multiplication or function. Additionally, certain HLA genes may enhance susceptibility to a virus that causes diabetes or may be connected to immune response genes that predispose people to an autoimmune reaction that is harmful to their own islet cells (autoaggression). When immunosuppressive medications like cyclosporine or azathioprine are administered at the first sign of type I diabetes, it appears that pancreatic B cell damage is lessened. This finding supports the idea that the immune system's auto-aggression is a significant contributor to the pathogenesis of this type of diabetes (Nolte and Karam, 2001).

Non-insulin dependent diabetes mellitus (NIDDM)

All other types of diabetes are considerably outnumbered by type II diabetes. Patients with NIDDM are not at risk for ketosis and are not dependent on exogenous insulin to prevent ketonuria. However, if fasting hyperglycemia cannot be controlled by diet or oral medications, they may need insulin, and under certain conditions, such as extreme stress brought on by illnesses or trauma, they may go into ketosis (Harris and Zimmet, 1997). The body does not properly utilize the insulin that the pancreas generates, which is the pathophysiology of type

II diabetes. The main cause of this is peripheral tissue insulin resistance, which occurs when insulin-receptors or other intermediates in the insulin signaling pathways within body cells become insensitive to insulin. As a result, glucose cannot easily enter the tissue, which causes hyperglycemia or elevated blood glucose levels (Albright, 1997). Most type II diabetes patients are obese, which generally results in decreased insulin action, and they will eventually need a variety of anti-diabetic medications to maintain sufficient glycaemic control (Nolte and Karan, 2001). (Gerich, 2001).

1.5.5 General symptom of Diabetes mellitus

- Increased thirst
- Frequent urination
- Weight loss
- Fatique
- Irritability
- Blurred vision
- Slow healing sores
- Frequent infection such as gums or skin infection and vaginal infections.

(WHO, 2013).

1.5.6 Pathophysiology of Diabetes Mellitus

With the exception of smooth muscle, where insulin operates through the IGF-1, insulin is the main hormone that controls the uptake of glucose from the blood into the majority of body cells, including the liver, adipose tissue, and muscle. Therefore, all types of diabetes mellitus are primarily caused by a lack of insulin or by the insensitivity of its receptors (Vijan, 2010). The breakdown of glycogen (glycogenolysis), the liver's storage form of glucose, intestinal absorption of meals, and gluconeogenesis, the body's process for producing glucose from

non-carbohydrate substrates, are the three main sources of glucose for the body. (2011) Shoback and Gardner In order to control the body's glucose levels, insulin is essential. Insulin can promote the transport of glucose into fat and muscle cells, as well as the storage of glucose in the form of glycogen. It can also inhibit the breakdown of glycogen or the gluconeogenic process (Shoback and Gardner, 2011). Beta cells (B-cells), which are found in the islets of Langerhans, release insulin into the blood. Langerhans in the pancreas, usually after eating, in response to increased blood glucose levels. About two-thirds of the body's cells utilize insulin to take glucose from the blood for use as fuel, as a starting material for other molecules that are needed, or for storage. Lower glucose levels cause the beta cells to release less insulin and cause the breakdown of glycogen into glucose. The hormone glucagon, which functions differently from insulin and is primarily responsible for controlling this process (Barrett, 2012). Because glucose is not correctly absorbed by the body cells that need it and is not properly stored in the liver and muscles if there is not enough insulin available, cells do not respond well to insulin's effects (insulin resistance), or the insulin itself is faulty (Shoback and Gardner, 2011). Overall, this leads to high blood glucose levels that don't go down, inadequate protein synthesis, and other metabolic abnormalities such metabolic acidosis in cases of severe insulin shortage. When the blood glucose level stays high for an extended period of time, the kidneys hit a reabsorption threshold and the body excretes glucose in the urine (glycosuria) (Murray, 2012). As a result, there is a rise in the osmotic pressure of the urine, increased polyuria, and greater fluid loss because the kidneys are unable to reabsorb water. Dehydration and increased thirst (polydipsia) are caused when lost blood volume is restored osmotically from water in body cells and other compartments (Shoback and Gardner, 2011). Additionally, low intracellular glucose increases hunger, which causes overeating (polyphagia).

1.5.7 Complications of Diabetes Mellitus

- Heart disease, heart attack and stroke
- Nephropathy
- Retinopathy and vision loss
- Hearing loss
- Depression
- Dementia

(Krishnasamy et al., 2018).

1.5.8 Management of diabetes mellitus

Physical training: in all types of diabetes, it reduces weight loss, central obesity, and blood lipids while improving insulin sensitivity, thus improving plasma glucose.

- Stop smoking
- Eat healthy food
- Insulin therapy
- Blood sugar monitoring (WHO, 2013).

1.6 MECHANISM OF ACTION

A group of disorders collectively known as diabetes mellitus (DM) occur when the body is unable to control the amount of sugar (more precisely, glucose) in the blood.

The body receives glucose from the blood to give it the energy it needs to carry out all of a person's everyday tasks. The liver produces glucose from the food a person eats. After that, the glucose is released into the circulation. In a healthy individual, various hormones, primarily insulin, control the blood glucose level. The pancreas, a little organ situated between the stomach and the liver, produces insulin. The pancreas also produces additional

vital digestive enzymes that are directly delivered into the gut. Glucose can go from the blood into the body's cells, where it can be used as fuel, thanks to insulin. Type 1 diabetes is characterized by insufficient insulin production, type 2 diabetes by improper insulin utilization, or both (which occurs with several forms of diabetes). Blood glucose levels remain high in diabetes because blood glucose cannot enter cells effectively. This damages some organs and tissues exposed to the high glucose levels in addition to starving all the cells that require glucose for nourishment. The perfect synchronization of endogenous glucose production or dietary glucose administration is necessary to maintain a normal plasma glucose levels. Three processes produce glucose: glycogenolysis, glucogenesis, and intestinal absorption that occurs after dietary carbs are digested (Sanders et al., 2016). Multiple metabolic routes are used to transfer glucose into cells. The liver and kidneys are the only organs that contain glucose-6-phosphatase, the enzyme required for the release of glucose into the circulation. It can be stored as glycogen, go through glycolysis to become pyruvate, and then be released into the circulation by these two organs. Plasma glucose levels are generally steady during a fast, indicating that the rates of glucose production and consumption are similar (Ramos an Juan 2017). Depending on the carbohydrate content of the meal and the rate and degree of glucose prouction absorption, the rate of exogenous glucose delivery into the circulation after a meal can be more than twice that of post absorptive endogenous glucose production. As glucose is absorbed, endogenous glucose production is reduced, and liver, muscle, and fat utilize glucose more quickly (Tracey et al., 2018). Exogenous glucose is subsequently absorbed, bringing the plasma glucose concentration back to a level that is similar to that of fasting. Higher amounts, such those found after meals, are necessary to boost glucose utilization; in the fasting state, it regulates the plasma glucose levels primarily by inhibiting hepatic glucose prouction (Ramos and Juan 2017).

1.6.1 HOW GLUCOSE CAUSES OBESITY

Ingestion of carbohyrate elicits a prompt rise in insulin concentration and decrease in glucagon concentration(Babashami et al., 2019). The increase in insulin concentrations, which occurs before the rise in arterial glucose concentrations , is thought to be mediated largely via hormonal signals arising in the gastrointenstinal tract (incretion effect) (Farb et al., 2016). The early insulin release alows increased glucose disposal during absorption and prevents hyperglycemia (Lebovitz an Harold 2016). The large change in glucose concentration that would result if absorption were not accompanied by an early increase in utilization would require much higher concentrations of insulin if the rise in insulin concentration occurred only after glucose entered the circulation (Islet dysfunction and peripheral insulin resistance are both present in type 2 diabetes and are both neccessary dfor the development of hyperglycemia). Glucagon concentrations increase as a result of insufficient insulin (secretion or action) (Wiviott et al .,2019). As the ratio between insulin and glucagon declines, the liver produces more glucose (basal hyperglycemia), whereas postprandial hyperglycemia is caused by a reduction in the absolute plasma insulin concentration or action (Lebovitz,Harold 2016).

1.6.2 STREPTOZOTOCIN-INDUCED ANIMAL MODELS OF DIABETES

The drug streptozotocin (STZ) is frequently used to cause experimental diabetes in mice (Ghamesi ,2014). Since its diabetogenic properties were first reported in 1963, STZ has been used alone, in combination with other chemicals, or with dietary manipulations to induce either type 1 or type 2 diabetes in rodents. Type 2 diabetes can be induced by at least three different methods, including STZ injection after nicotinamie administration, high fat diet (HFD) feeding followed by a low-dose STZ injection are perhaps able to reduce blood sugar levels (Patil,2014).

1.6.3 MECHANISMS OF STZ ACTION

The bacterium Streptomyces achromogens produces the antibiotic STZ, which has a wide range of antibacterial activities (Eleazu , 2013). a highly reactive methylnitrosourea moiety that is through to exert STZ's cytotoxic effects, while the glucose moiety directs the chemical to the pancreatic beta-cells. It also contains a glucose molecule (in deoxy form). The GLUT2 receptor, which is absent from the plasma membranes of beta cells, is recognized by STZ (Eleazu, 2013). Therefore, STZ specifically targets pancreatic beta-cell. Due to the lesser amount that GLUT2 also exists in the liver and kidney, excessive doses of STZ may also have an adverse effect on liver function and kidney. STZ has a really short life (with a half-life of 15 minutes in the serum after IV injection); therefore, its acute toxicity to the liver and the kidney can be neglected after persistent hyperglycemia is achieved. STZ is rapidly metabolized in the liver and quickly eliminated by renal excretion after ingestion (Bouwens, 2005). (Tesch,2007). Any subsequent functional impairment of the liver and kidney after STZ has been removed from the body may be attributable to the consequences of diabetic hyperglycemia. This is the foundation for understanding how STZ diabetes problems affect these organs as well as other organs like the brain, the heart, and the muscles (Tesch,2007).

METFORMIN

The main purpose of metformin is to treat type 2 diabetes, especially in obese patients. Metformin has demonstrated a reduction in diabetes-related complications and death of roughly 30% when compared to insulin, glibenclamide, and chloropropamide (Rafieian-Kopaei, 2013). Metformin lowers blood levels in a variety of ways, including through nonpancreatic mechanisms that don't result in an increase in insulin secretion. Because it enhances the effects of insulin, it is known as an insulin sensitizer. Metformin also inhibits the liver's ability to produce endogenous glucose, primarily as a result of a slowing down of

glyconeogenesis and a negligible impact on glycogenolysis. The increased non-oxidative glucose disposal into the skeletal muscle caused by metformin enhances peripheral glucose disposal. It typically does not result in hypoglycemia, which makes it a special anti-diabetic medication. (Rosen, 2006).

In comparison to insulin and sulfonylureas, metformin therapy for diabetes is associated with a lower risk of weight gain. Gaining weight improves glucose regulation. According to a study, patients treated with glibenclamide gained roughly 3 kg over the course of a 10-year course of treatment, whereas those treated with insulin gained 6 kg (impact of intensive blood glucose control with metformin on complications in overweight patients with type 2 diabetes).

CHAPTER TWO

MATERIALS AND METHOD

2.0 MATERIALS

The following materials were used during the research study;

- Nose mask
- Methylated spirit
- Gongronema latifolium
- Cotton wool
- Gloves
- Measuring cylinder
- Glass rod
- Steel plates
- Ceramic plates
- Cages
- Wistar rats
- Aluminium foil
- Buckets
- Handkerchief
- Test strips
- EDTA container
- Masking tape
- Universal bottle
- Blade and scissors
- Seperating funnel
- Glass jar

- Lancet
- digital weighing balance
- Spatula
- pH meter
- Syringe
- Centrifuge
- Plain container
- Sensitive weighing balance
- Oral gavage
- mortal and pestle
- Dessecting set
- Test tube racks
- 250ml and 1000ml beakers
- Microcentrifuge tube

2.1 CHEMICALS AND REAGENTS USED

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

- DCM
- Ethanol
- N-hexane
- Picric acid
- Tween-80
- Picric acid
- Chloroform
- Cholesterol reagent kit

- Triglycerides reagent kit
- Total protein reagent kit
- HDL-cholesterol reagent kit

2.1.1 METHODOLOGY

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

2.2.1 PREPARATION OF PLANT EXRACTS

The crushed was steeped in hyroethanol in a jar containing 800 ml of ethanol and 200 ml of distilled water in an 80:20 ratio. A glass rod was used to swirl the mixture until it was free. After being let to stand for seven days, the marc was sieved using a white, unused handkerchief to remove it from the crude extract. The obtained crude extract was put into tidy jars with labels. The marc was once more immersed in ethanol for three more days in a jar using the same 80:20 ethanol to distilled water ratio. Exhaustive extraction is the name given to this procedure. Then, in a 24-hour period, the crude extract was delivered to the pharmaceutical chemistry staff research lab for freeze drying.

2.2.2 FRACTIONATION

The crude extract was dissolved in distilled water. A separating funnel was used to receive the mixture. DCM was added to the mixture, which was then thoroughly agitated (shaken). The separation funnel was left standing for around an hour. The DCM was then poured into a glass jar and delivered to a pharmaceutical Chemistry research facility for freeze drying after the crude extract had been totally allowed to drain out.

2.2.3 PREPARATION OF THE ANIMALS USED

Male wistar rats were obtained when they were 16 weeks old, housed in tidy, tranquil cages, and allowed to acclimate for two weeks by being fed regularly with grower mash, a common poultry feed. The wistar rats were divided into fourteen (14) groups after acclimatization, with seven animals kept in a separate cage for each group. The rats were then each dyed with picric acid on various body parts including their tails, heads, legs, hands, and backs before being weighed on a weighing balance. This served as identification.

2.2.4 EXPERIMENTAL DESIGN

The male wistar rats were arranged into eight (8) groups which are:

Group one: This is the standard control; they were given only water for the course of the study's 14 days and were fed a standard meal (100 mg). No type of treatment was given, and no diabetes mellitus was induced.

• Groups Two: These groups served as the diabetes control; they consumed a high-fat diet (100 mg) and only water for 14days.

Streptozotocin was used to induce diabetes mellitus, and no therapy was given for the entire investigation.

Group Four: For 14 days, they were only given water and a high-fat diet. Streptozotocin was used to induce diabetes mellitus, along with the commonly used diabetic mellitus medication metformin (500g), which was given orally throughout the course of the trial.

• Group Five: For 14 days, they were given a high-fat diet (100 mg) and just water. Streptozotocin was used to induce diabetes mellitus, and daily oral doses of 200 mg of *Gongronema latifolium* extract were given throughout the course of the trial.

• Group Six: For 14days, they were given a high-fat diet (100 mg) and just water. Streptozotocin was used to create diabetes mellitus, and daily oral doses of 400 mg of *Gongronema latifolium* extract were given throughout the course of the trial.

• Group Nine: For 14 days, they only received water and (100mg) of food. Streptozotocin was used to generate diabetes mellitus, and DCM fraction of *Gongronema latifolium* (200 mg) was given orally every day for the duration of the trial.

Group 10: For 14 days, they only received water and (100mg) of food. Streptozotocin was used to generate diabetes mellitus, and DCM fraction of *Gongronema latifolium* (400 mg) was given orally every day for the duration of the trial.

• Group Eleven: For only 14 days, they were given a high-fat diet (100 mg) and only water. Streptozotocin was used to induce diabetes mellitus, and N-hexane of *G. latifolium* (200 mg) was given orally throughout the study day.

• Group Twelve: For only 14 days, they were given a high-fat diet (100 mg) and water. Streptozotocin was used to produce diabetes mellitus, and N-hexane of *Gongronema latifolium* (400 mg) was given orally every day for the length of the study.

2.2.5 TREATMENT OF THE ANIMALS WITH THE EXTRACT

After three days of STZ induction, the rats were fasted overnight to measure their fasting blood glucose levels and determine whether they were diabetic. The dosages for two groups of wistar rats were 200 mg of the n-hexane fraction of G. latifolium per kg of rat body weight and 400 mg of the n-hexane fraction of G. latifolium for each rat, respectively.

2.3.0 SACRIFICING THE ANIMALS

A small piece of cotton wool was soaked in chloroform and placed in a beaker. The next step was to place each rat inside the beaker in order to give them consciousness. Then they were

taken outside and dissected. They were given a blood draw with a syringe, and the blood was kept in an EDTA container. other crucial organs like the pancreas, liver, and kidney.

2.3.1 FRACTIONATION USING DCM AND N-HEXANE

The solvent to solvent partitioning principle was used in this separation process, which was carried out using a separating funnel. After freeze-drying, the marc was measured, combined with distilled water, stirred, and completely dissolved. Ethanol was then added to aid in the process. 80:20 was the ethanol to water ratio. DCM and n-Hexane were added to the marc after it had been poured into the separating funnel in order to obtain the DCM fraction. The DCM separates the marc, which drains out through the valve leaving behind the less dense DCM fraction, and collects the polar components from it. A layer of DCM fraction was formed above and was collected by doing this. Until a colorless layer appeared showing that the DCM had entirely removed the polar components from the marc, this process was repeated every hour. The separated marc was refilled and the remaining ingredients were collected by adding n-Hexane to the mixture. Pharmaceutical Chemistry Research received the DCM and hydro-ethanol fraction for freeze drying.

2.3.2 INDUCING OF DIABETES MELLITUS USING STREPTOZOTOCIN

The animals were fed for 28 days while having their weight checked on a weekly basis until they became fixated on it. An insulin syringe was used to give the streptozotocin medication intraperitoneally, and blood samples were taken from the wistar's tail to evaluate the wistar's fasting blood glucose levels three days later. And a rat wasn't considered diabetic until its fasting blood sugar level was more than 200 mg/dl.

2.3.2 PREPARATION OF STREPTOZOTOCIN (STZ) TO BE ADMINISTERED

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

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

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

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

2.3.3 PREPARATION OF COLD BUFFER

2.989g of sodium citrate and 2.1g of citric acid was weighed.

It was then combined with 50 ml of cold distilled water to dissolve it, and it was then transferred to a measuring cylinder and filled to the top with 100 ml of cold distilled water. At 4.5, the pH was measured. The solution had a slight acidity to let the streptozotocin's damaging effects on the animal's pancreas, which caused diabetes mellitus. A basic, potassium hydroxide, was used to restore the pH after it had decreased due to environmental factors.

2.4.0 FASTING BLOOD SUGAR

The wistar rats were starved for the whole night before having their tail tips blooded. After cleaning the tail tip with methylated spirit and pricking it with a Lancet, two drops of blood

were placed on the glucose test strip and put into the glucometer device. After 15 seconds, the blood glucose level was displayed on the LED screen in mg/dl.

Normally, all of this is finished before 10:00 am. As soon as diabetes was induced, this was typically done every three days.

2.5.0 PREPARATION OF STANDARD TREATMENT DRUG TO BE ADMINISTERED

The 500mg metformin pills were broken into powder using a mortar and pestle, then added to 10ml of distilled water and mixed until thoroughly dissolved. The amount that has to be given depends on the weight of the wistar rat.

2.6 EUTHANIZATION AND SAMPLE COLLECTION FROM WISTAR RATS

The wistar rats were euthanized after receiving the standard medication (metformin), N-hexane extracts, and Ethyl acetate extracts for 14 days. Then, blood and organ samples were taken from each animal. Last but not least, the fasting blood glucose was measured. The rats were then put into a container with cotton balls that had been soaked in chloroform. After a short while, the rats would pass out. Immediately, the rats were inverted, and a dissecting blade was used to cut open a section of the perineum cavity and extend it toward the thoracic cavity of the wistar rat. Blood was drawn, put into micro centrifuge tubes, EDTA containers, lithium heparin containers, and containers with bold labels that matched the labels of each animal.

Additionally, organ samples from the liver, pancreas, and kidney were obtained, put in test tubes with formalin preservation, and kept in a freezer. The wistar rats received food for 11 weeks before being treated for diabetes.

2.6.1 HOMOGENIZATION

100g of phosphate buffered saline was dissolved in 200ml of distilled water. 10ml of the solution was taken and put into a fresh mortar and pestle.

The liver was brought out to the mortar and pestle along with the buffer solution and pounded until smooth for each test tube that had been set on ice. Once smooth, the homogenates were transferred into the appropriate test tubes and stored in the refrigerator.

CHAPTER 3

RESULTS

Effect of Aqueous fraction of *G. latifolium stem bark* on Body Weight Changes (g) in Streptozotocin Induced Diabetic Rats

The results show that rats induced with streptozotocin significantly decrease the weight of the rats (weight loss) when compared to the normal control, but administration of aqueous fraction of of G. *latifolium stem bark* to Wistar rats for 14 days showed a steady significant increase in body weight in a dose dependent manner compared to the diabetic control (Table 1). Oral administration of metformin significantly increases the weight of the rats after the 14 days period.

Table 1: Effect of Aqueous fraction of *G. latifolium stem bark* on Body on Body Weight Changes (g) in Streptozotocin Induced Diabetic Rats

Groups	Initial Body Weight (g)	Final Body Weight(g)	Weight Loss/Gain(g)
Group 1	190.48±1.56	204.47±5.08	+13.99±0.14
Group 2	184.10±1.12	162.66±2.66	-21.44±0.11
Group 3	167.42±1.58	185.55±2.09	+18.13±0.18
Group 4	212.24±2.60	198.52±2.11	-13.72±0.15
Group 5	182.20±1.80	194.11±1.29	+11.91±0.22

Data were expressed as mean ± SEM, n=6. Group 1, Normal control, Group 2, Diabetic control (STZ only), Group 3, Positive control (Metformin), Group 4, Aqueous fraction (200 mg/kg bw), Group 5, Aqueous fraction (400 mg/kg)

Effect of Aqueous fraction of *G. latifolium* stem bark on Fasting Blood glucose in Streptozotocin Induced Diabetic Rats

The effect of aqueous fraction of *G. latifolium* stem bark on fasting blood glucose in STZ induced diabetic rats is shown in Fig 1. The result indicates that the blood glucose level of the diabetic control and the diabetic treated increased significantly ($p < 0.05$) when compared to

the normal control three days after administration of streptozotocin. At the same time, it normalized in the diabetic treated rats fourteen days after the administration of aqueous fraction of *G. latifolium* stem bark when compared with the normal control. The glucose level of the diabetic control remained significantly increased ($p < 0.05$) compared with the normal control and diabetic treated after the fourteen days period of study

Table 2: Effect of Aqueous fraction of *G. latifolium* stem bark on Fasting Blood glucose in Streptozotocin Induced Diabetic Rats

Group/Treatment	Glucose (mg/dl) Day 0	Glucose (mg/dl) Day 3 (After induction)	Glucose (mg/dl) Day 7	Glucose (mg/dl) Day 14
Group 1	85.40±2.34[a]	86.20±2.40[a]	84.10±1.34[a]	84.40±2.50[a]
Group 2	88.20±3.24[a]	*460.00±11.43[b]	*318.25±5.20[c]	*348.10±10.20[d]
Group 3	80.24±4.34[a]	**337.25±14.50[b]	**111.32±0.63[c]	**108.50±6.20[c]
Group 4	86.80±3.20[a]	**220.20±8.12[b]	**204.46±10.19[c]	**160.20±1.25[d]
Group 5	82.60±4.14[a]	**248.40±4.00[b]	**207.87±8.20[c]	**150.12±2.50[d]

Data were expressed as mean ± SEM, n=6. Values with different alphabet are statistically significant ($p < 0.05$). *Mean is significant ($p < 0.05$) when compared with the control. ** mean is significant ($p < 0.05$) when compared with diabetic control group. Group 1, Normal control, Group 2, Diabetic control (STZ only), Group 3, Positive control (Metformin), Group 4, Aqueous fraction (200 mg/kg bw), Group 5, Aqueous fraction (400 mg/kg)

Effect of aqueous fraction of *G. latifolium* stem bark on creatinine levels in streptozotocin induced diabetic rats

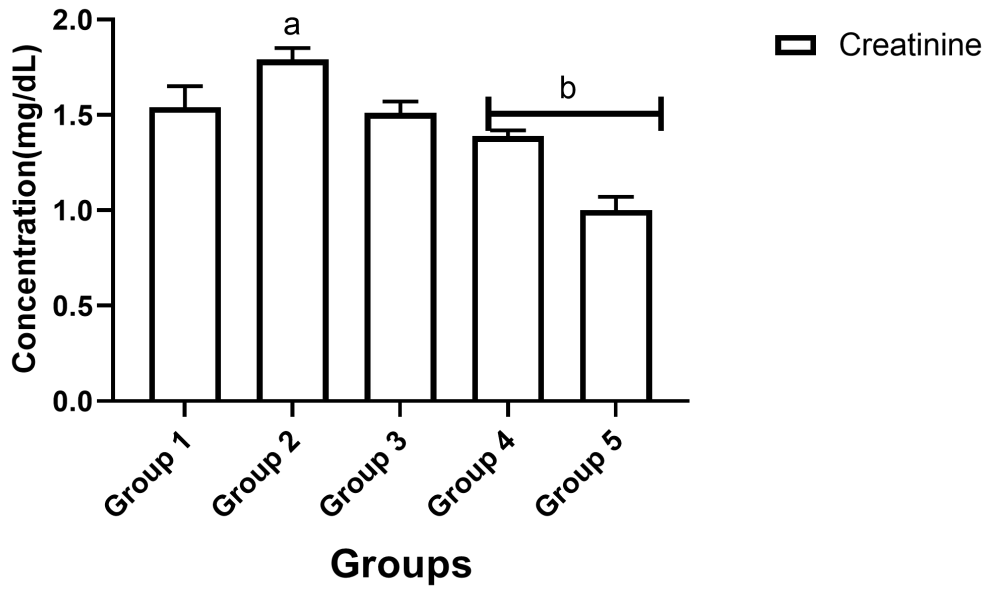

Fig 1: Effect of aqueous fraction of *G. latifolium* stem bark on creatinine levels in streptozotocin induced diabetic rats. Data were expressed as mean ± SEM, n=6. Values with different alphabet are statistically significant $p < 0.05$) Group 1, Normal control, Group 2, Diabetic control (STZ only), Group 3, Positive control (Metformin), Group 4, aqueous fraction (200 mg/kg bw), Group 5, aqueous fraction (400 mg/kg)

Effect of aqueous fraction of *G. latifolium* stem bark on urea levels in streptozotocin induced diabetic rats

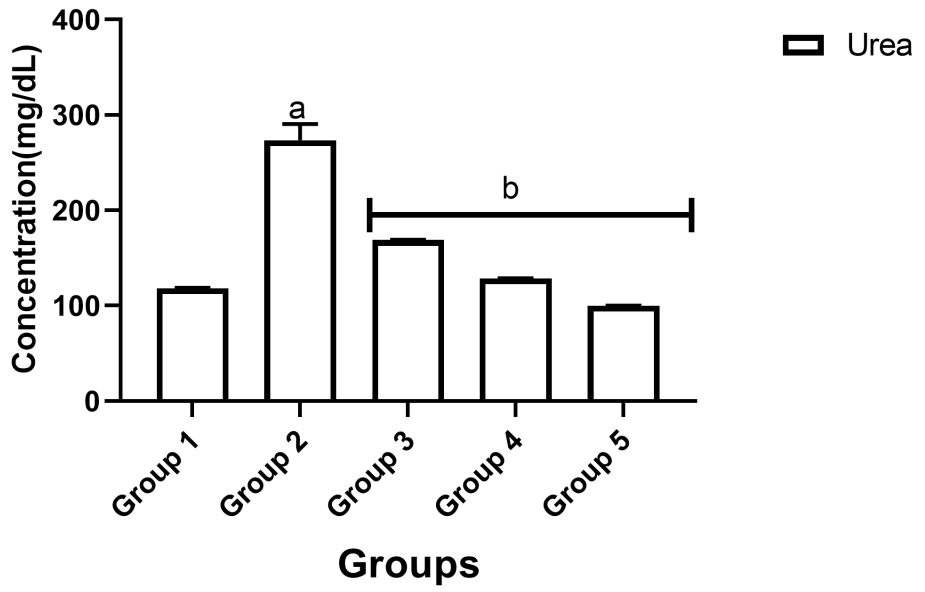

Fig 2: Effect of aqueous fraction of *G. latifolium* stem bark on urea levels in streptozotocin induced diabetic rats. Data were expressed as mean ± SEM, n=6. Values with different alphabet are statistically significant $p < 0.05$) Group 1, Normal control, Group 2, Diabetic control (STZ only), Group 3, Positive control (Metformin), Group 4, aqueous fraction (200 mg/kg bw), Group 5, aqueous fraction (400 mg/kg)

CHAPTER FOUR

DISCUSSION AND CONCLUSION

Chemical streptozotocin significantly reduces the body weight of rats, whereas metformin and DCM extract of Gl *(Gongronema latifolium*) stem back significantly raise body weight in rats. This suggests that the effects of streptozotocin and DCM on rat weight are diametrically opposed. Numerous variables may have contributed to the weight loss and elevated blood glucose levels seen in the streptozotocin-induced rats. One explanation is that streptozotocin, a powerfully diabetogenic substance, harms the pancreatic beta-cells that produce insulin, impairing their ability to produce insulin, which results in weight loss and a rise in blood sugar levels.

On the other hand, their capacity to control blood sugar levels and enhance insulin sensitivity may account for the increase in body weight and decrease in blood glucose levels seen in the rat treated with DCM extract of *G. latifolium* stem bark and metformin. The results indicate that *G. latifolium* stem bark extracts STZ and DCM both have a significant impact on rats' body weight and blood glucose levels, with STZ causing weight loss and an increase in blood glucose levels and DCM extract causing weight gain and a decrease in blood glucose levels. Metfomin seems to have a beneficial impact on rats' body weight and blood sugar levels.

In STZ-induced diabetic rats, the DCM fraction has a positive effect on creatine levels. The waste product creatinine is normally eliminated from the body through the kidneys. It is produced when muscle tissue breaks down. In STZ-induced diabetic rats, therapy with the stem bark of GL can considerably lower creatinine, according to several research.

CONCLUSION

The given plant extract caused a decrease in kidney enzyme activity levels. This study has shown that *gongronema latifolium* has good potential for kidney protection, hence it may be said that.

The findings of the current study strongly suggested that DCM fraction from *Gongronema latifolium* stems be used for its considerable hypoglycemic action in the treatment of diabetes.

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APPENDIX

Rats induction of STZ

1. To calculate for the Mg of STZ dose (30mg/kg of STZ)

30mg=1kg

30mg=1000g

x=206.81g

x= 206.81 x 30 /1000

x= 6.20g

2. To calculate the buffer volume

2ml is the assumed bulk volume

(1ml can also be used)

2ml=1000g

x=206.81g

x=206.81g x 2/1000

X=0.41ml

3. To calculate buck STZ weight and buffer volume

6.20mg x 96= 0.41ml x 96

596.2mg = 39.36ml

4. To calculate volume for a particular rat from bulk calculation (using 6.20 as mg of dose)

595.2mg= 39.36ml

6.20mg= X

X=6.20 x 39.36/595.2mg

x =0.41ml

NB: Rats won't take more than 1ml IP (Intraperitorial) and more than 5ml orally

GROUP 4 (200mg/kg of Metformin)

	Weight	Dose in mg	Volume of dose(ml)
Head	185.25	37.06	1.85
	Weight	Dose in mg	Volume of dose(ml)
Head	185.25	37.06	1.85
Back	171.97	34.39	1.72
Right hand	180.02	36.00	1.80
Tail	181.63	36.33	1.82
Right leg	171.95	34.39	1.72
Total		178.17	8.91

Stock solutions

178.17mg —— 8.91ml

500mg ——— x

X= 8.91 x 500/178.17

X=25.00ml

Stock Concentration

500mg -----25ml

X ----- 1ml

X= 500x1/25

X= 20mg/ml