HEPATOPROTECTIVE POTENTIAL OF METHANOL EXTRACT OF *GONGRONEMA LATIFOLIUM* STEM BARK IN STREPTOZOTOCIN INDUCED DIABETIC WISTAR RATS

BY

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CERTIFICATION

We the undersigned hereby certify that Miss Tina Chika Okoye (BMS1702033) carried out this work in the Department of Medical Biochemistry, University of Benin, Benin City and we approve same as adequate in scope and quality for the award of Bachelors of Science Degree (B.Sc) in Medical Biochemistry.

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DEDICATION

I dedicate this project work to God Almighty my creator, my strong pillar, my source of inspiration, wisdom, knowledge and understanding. He has been the source of my strength throughout this program and on His wings only have I soared. I also dedicate this work to my dad Mr. Emeka Okoye, whose love and care has nurtured me all the way and whose encouragement has made sure that I give all it takes to finish that which I have started.

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ABSTRACT

Aqueous extract of *Gongronema latifolium* was examined for its hepatoprotective properties. For a week, 98 male Wistar rats—which weigh between 120 and 150g each—were kept in orderly, quiet cages and given access to grower mash as needed to aid in their acclimatization. Groups of rats were separated out. The weight range of each group member was typical for the entire group. As a result, at the start of the experimental period, the rats had reached an average weight of 150–200g. Animals in Group 1 just got food and water as a standard control. In groups 2, 3, 4, and 5, diabetes was produced. Streptozotocin was used to stimulate the group 2 rats, however they were not given any medication. Streptozotocin was used to stimulate group 2 rats, but they were not given any further treatment, making them the negative control group. Metformin was used to initiate and treat the group 3 rats. Group 4 rats received 200mg/kg body weight of the bvaqueous fraction, and group 5 received 400mg/kg of the aqueous fraction. The results of the fasting blood glucose levels (mg/dl) of the rats in each group were: group 1, 84.40±2.50a, group 2, 348.10±10.20d, group 3, 108.50±6.20c, group 4, 160.20±1.25d, group 5, 150.12±2.50d, for each group respectively. The average body weights of the rats in each group were: group 1, 13.99±0.14, group 2, -21.44±0.11, group 3, 18.13±0.18, group 4, -13.72±0.11, group 5, 11.91±0.22. *Gongronema latifolium* significantly (p0.05) decreased fasting blood glucose level, raised albumin level, decreased ALT and AST level, and increased body weight in rats when administered at doses of 200 mg/kg body weight and 400 mg/kg aqueous fraction, respectively.

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

In the past ten years, there has been a resurgence of interest in wild fruits as a means of supplying food, nutrition, and income (Maroyi, 2012; Maroyi and CheikhYoussef, 2017). Given that these fruits include a variety of phytochemicals and micronutrients that are crucial for human nutrition and health. A significant part of these wild fruits is classified as both foods and medicines based on evidence acquired over time (Lako *et al*., 2007; Lamien-Meda *et al*., 2008). These wild edible fruits have medicinal properties, including antioxidant effects that counteract oxidative stress, making them useful in the prevention of chronic illnesses such as heart disease, cancer, diabetes, hypertension, stroke, and Alzheimer's disease (Lako *et al*., 2007). Wild edible fruits have been found to have exceptional nutritional qualities such as an excellent mineral source, fiber, vitamins, polyphenols, ascorbic acid, and fatty acids, all of which add flavor and color to the diet (Glew *et al*., 2005; Lamien-Meda *et al*., 2008).

Some of these wild edible fruits are used to treat a variety of illnesses in trado-medicine. This is most likely due to herbal medicines' efficacy, availability, and cost effectiveness. They can also be used as food supplements to supplement a poor diet.

Diabetes Mellitus (DM) is one of the most serious global health issues, posing a significant burden on public health as well as social and economic development, and is a leading cause of death worldwide. According to the International Diabetes Federation (IDF), **451** million people worldwide suffer from diabetes, with the number expected to rise to **693** million by **2045** if the disease is not controlled (Cho *et al*., 2018). Diabetes mellitus is a metabolic disorder with multiple etiologies in which a person has high blood glucose levels due to insufficient insulin production or an inability of body cells to respond properly to insulin, resulting in the destruction of carbohydrate, fat, and protein metabolism (Hana and Adma, 2017).

Type 1 diabetes and type 2 diabetes are the two most common types of diabetes. T1DM is also referred to as insulin-dependent diabetes. It is characterized by insufficient insulin production in

the body and is caused primarily by pancreatic islet beta cell destruction (Salsasi and Nathan, **2006**). Patients with T1DM are at risk of ketoacidosis and require daily insulin administration to control the amount of glucose in their blood. T1DM is most common in children and adolescents (Folorunso and Oguntibeju, **2013**). T2DM, also known as non-insulin-dependent diabetes, occurs as a result of the body's insufficient use of insulin, which can result in hyperglycemia (Spellman, 2010). Hyperglycemia has been linked to oxidative stress in diabetes by interfering with the electron transport chain, resulting in an excess of superoxide anions, which can harm a variety of tissues (Snowling and Hopkin, 2006) T2DM accounts for the vast majority of diabetes cases worldwide. Insulin resistance is caused by a decrease in the responsiveness of target tissues to normal circulating insulin levels (Tripathy and Chavez, 2010). Family history of diabetes, older age, overweight, obesity, unhealthy diet, physical inactivity, excessive alcohol consumption, and smoking are all risk factors for diabetes. T2DM affects the vast majority of diabetics (90%); it typically affects adults but, in recent years, has also been found in children).

It is expected that drugs will play a role in the treatment of these diseases; however, the majority of the synthetic drugs available for the treatment of diabetes have severe side effects as well as other disadvantages (Tahrani *et al*., 2010). According to studies, medicinal plants have played a dominant role in the healthcare system, particularly in developing countries where herbal medicine has a long history of use (Bhowmik *et al*., 2012). According to reports, the majority of the population prefers traditional medicine to conventional drug treatment for primary health care. Traditional medicine may be used due to the abundance of potential health benefits of plants, as well as their efficacy, with little or no side effects, and safety.Medicinal plants have been used for health maintenance by almost all cultures. The widespread use of traditional herbs and medicinal plants as remedies for diseases can be traced back to the presence of bioactive compounds in such plants, which have medicinal potency (Jomoh *et al.,* 2017).

1.2 JUSTIFICATION OF STUDY

The present study is to validate the claim that methanol extract of *Gongronema latifolium* 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 ethnomedicine.

1.3 AIM OF STUDY

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

1.4 OBJECTIVES OF STUDY

The following are the objectives of this study are:

To determine its ameliorating potential to manage diabetic related complication.

To evaluate the effects of methanol extract of *Gongronema latifolium* on diabetes induced rats in relation to:

kidney function

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 ETYMOLOGY AND HISTORY OF DIABETES

Diabetes is derived from the Greek word "Diabetes," which means "to siphon or pass through," and the Latin word "mellitus" (honey or sweet). Aretus the Cappadocia described diabetes as passing too much water (polyuria). In 1675, Thomas Willis added mellitus, which he derived from the term "Mel," which means "honey," in recognition of the fact that diabetics' blood and urine contain an excess of glucose. DM can literally be translated as "siphoning" sweet water. The term "Sweet Urine Disease" was coined after ants were observed to be attracted to some people's urine due to the presence of glucose in the urine (Mandal, 2012). Diabetes is associated with the destruction of pancreatic -cells, which results in insulin deficiency and insulin resistance (American Diabetes Association, 2010).

Diabetes, regardless of type, is caused by having too much glucose circulating in your bloodstream. However, the cause of your high blood glucose levels varies depending on the type of diabetes. Diabetes mellitus is a metabolic disorder characterized by hyperglycemia caused by faulty insulin secretion, faulty insulin action, or both. Diabetes' chronic hyperglycemia is linked to a number of long-term microvascular complications affecting the eyes, kidneys, and nerves, as well as an increased risk of cardiovascular disease (CVD) (American Diabetes Association, 2012). Hyperglycemia causes oxidative stress through a variety of mechanisms, including an increase in the production of advanced glycated end products (AGEs), superoxide radicals, protein glycosylation, inflammatory mediators, and glucose autoxidation (Garay-Sevilla et al., 2005; Matsuda and shimomura, 2013).

As a result, when ROS is overproduced, it outnumbers the antioxidant capacity system's ability to counterbalance and eliminate these species, resulting in oxidative stress (Rahman et al., 2012). The oxidative stress in diabetes is caused by the autoxidation of glucose levels, which usually results in the generation of free radicals and the destruction of cellular homeostasis (Khan et al., 2015). Diabetes on the other hand, is a serious, long-term condition that has a significant negative impact on people's lives all over the world. It is one of the top ten causes of death in

adults, with an estimated four million deaths worldwide in 2017. (International Diabetes Federation, 2017). The prevalence of type 1 and type 2 diabetes in children and adolescents has also increased, with estimates of over one million children and adolescents under the age of 20 suffering from type 1 diabetes (Debelea *et al*., 2014). High blood glucose levels characterize this disease. Diabetes that is uncontrolled increases the risk of cardiovascular disease, stroke, and nerve damage, foot ulcers that lead to lower limb amputations, kidney failure, blindness, and premature death (World Health Organization, 2016). These conditions are responsible for more than 80% of all premature noncommunicable disease (NCD) deaths (Forouzanfar *et al*., 2016). Diabetes patients have a 2-3-fold increased risk of death from any cause (Yang *et al*., 2019). Diabetes necessitates lifestyle changes such as weight management, physical activity, and an appropriate diet, as well as medication use, in order to maintain normal glycemia and reduce the risk of long-term complications (Bagnasco *et al*., 2014).

Furthermore, despite progress in promoting population health and extending life expectancy, diabetes is the second most significant negative total effect on reducing global health adjusted life expectancy worldwide (Chen *et al.*, 2019).

Diabetes prevalence in Sub-Saharan Africa is rapidly increasing, mirroring global trends. Sub-Saharan Africa is expected to have the highest percentage increase in diabetes incidence of any region in the world. In 2015, the International Diabetes Federation (IDF) estimated that 14.2 million people in Sub-Saharan Africa had diabetes. It is highly variable, with significant differences in rural and urban prevalence, with urban areas bearing the brunt of the burden. The wide variation in diabetes prevalence can be attributed in part to regional differences in lifestyle and BMI. If nothing is done, this figure will rise by 162% by 2045. Africa has the highest percentage of undiagnosed people - 70% of diabetics are unaware that they have the disease. Diabetes was responsible for approximately 312,000 deaths in 2017, with people under the age of 60 accounting for three out of every four (73%) deaths.

This increase is the result of aging populations and lifestyle changes, such as unhealthy diets and a lack of physical activity. Obesity and being overweight are major risk factors for T2DM (International Diabetes Federation, 2019). The current prevalence of diabetes in adults aged 20 to 69 years in Nigeria is reported to be 1.7%. (International Diabetes Federation, 2017). In Nigeria,

approximately 105,091 diabetes-related deaths were reported in 2013. (Oputa and Chinenye, 2015). Recently, a pooled diabetes mellitus prevalence of 5.77% was reported, implying that 11.2 million Nigerians (1 out of every 17 adults) have the disease (Uloko *et al*., 2018).

Diabetes is also becoming more prevalent in Africa, with the African region expected to experience the greatest percentage increase (143%) in diabetes burden between 2019 and 2045. (International Diabetes Federation, 2018). Similarly, the prevalence of diabetes mellitus has steadily increased in Nigeria. Diabetes mellitus prevalence rates in some rural communities of Nigeria have been reported to range between 0.8 and 4.4 percent (Sabir et al., 2013), (Oladapo et al., 2010), while the prevalence in urban areas has not been reported.

Between 4.6 and 7% (Sabir *et al*., 2011), (Enang *et al.,* 2014). A recent systematic review and meta-analysis of studies on the prevalence of diabetes mellitus among Nigerians found an overall pooled prevalence of 5.77%. (Uloko *et al*., 2018). As of 2019, it was estimated that 8.2 million Nigerians had impaired glucose tolerance, with the figure expected to rise to 11.5 million by 2030. 2018 (International Diabetes Federation). This is extremely concerning because impaired glucose tolerance is a major risk factor for the development of type 2 diabetes and cardiovascular disease in the future. Diabetes mellitus is set to become an even bigger health problem in Nigeria as urbanization continues and populations age (Aisha et al., 2011).

2.2 TYPES OF DIABETES

2.2.1 Type 1 Diabetes

This type of diabetes, which accounts for only 5-10% of those with diabetes previously referred to as insulin-dependent diabetes or juvenile-onset diabetes, is caused by a cellular-mediated autoimmune destruction of pancreatic -cells, which most commonly occurs in children; however, type 1 diabetes can develop in adults. In type 1 diabetes, the body is unable to produce insulin or enough insulin because the immune system has attacked and destroyed the cells that produce insulin (pancreatic beta cells) (Michael *et al*., 2013). Type 1 diabetes occurs when an individual's immune system destroys insulin-producing beta cells. (Suresh and Lal, 2016). Monogenic diabetes refers to rare forms of diabetes caused by mutations or changes in a single gene. The two most common types of monogenic diabetes in young people are Neonatal Diabetes Mellitus

(NDM) and Maturity-Onset Diabetes of the Young (MODY) (Rubio-Cabezas et al., 2014). Diabetes that occurs before the age of 6 months is more likely to be non-autoimmune type 1 diabetes mellitus than autoimmune type 1 diabetes mellitus (T1DM). MODY is a group of autosomal-dominant inherited disorders in which hyperglycemia occurs at a young age and is usually mild. Rather than insulin resistance, it develops from beta cell dysfunction. In addition to the typical young people with acute onset T1DM, there is an older group with obvious type 2 diabetes (T2DM) but no evidence of insulin resistance. Aside from the typical young people with acute onset T1DM, there is an older group with apparent type 2 diabetes (T2DM) but evidence of autoimmunity as measured by Anti-Glutamic Acid Decarboxylase GAD antibody) measurements and eventually become insulin dependent. This is referred to as latent autoimmune Diabetes of Adults (LADA) (Richard *et al*., 2010).

2.2.2 Type 2 Diabetes

This type of diabetes, which affects 90-95% of those with diabetes and is also known as non-insulin-dependent diabetes or adult-onset diabetes, can affect people of any age, including children. However, type 2 diabetes is most common in people in their forties and fifties. Overweight and inactive people are also more likely to develop type 2 diabetes. It usually starts with insulin resistance, which occurs when fat, muscle, and liver cells do not use insulin to transport glucose into the body's cells for energy use. As a result, the body requires more insulin to assist glucose entry into cells. Initially, the pancreas responds to the increased demand by producing more insulin. When blood sugar levels rise, such as after meals, the pancreas produces insufficient insulin over time. Type 2 diabetes occurs when the pancreas no longer produces enough insulin; these people do not require insulin treatment to survive (Michael Parchman and colleagues, 2013).

2.3 Diabetes during pregnancy

When a woman is pregnant, she may develop gestational diabetes. Pregnant women produce hormones that can contribute to insulin resistance. Later in pregnancy, all women develop insulin resistance (Plow *et al*., 2018). Gestational diabetes develops when the pancreas does not produce enough insulin during pregnancy (Hartling *et al.,* 2012). Women who are overweight or obese

are more likely to develop gestational diabetes. It usually disappears after the baby is born. A woman who has had gestational diabetes, on the other hand, is more likely to develop type 2 diabetes later in life. Babies born to diabetic mothers are also more likely to develop obesity and type 2 diabetes (Zhu *et al*., 2016).

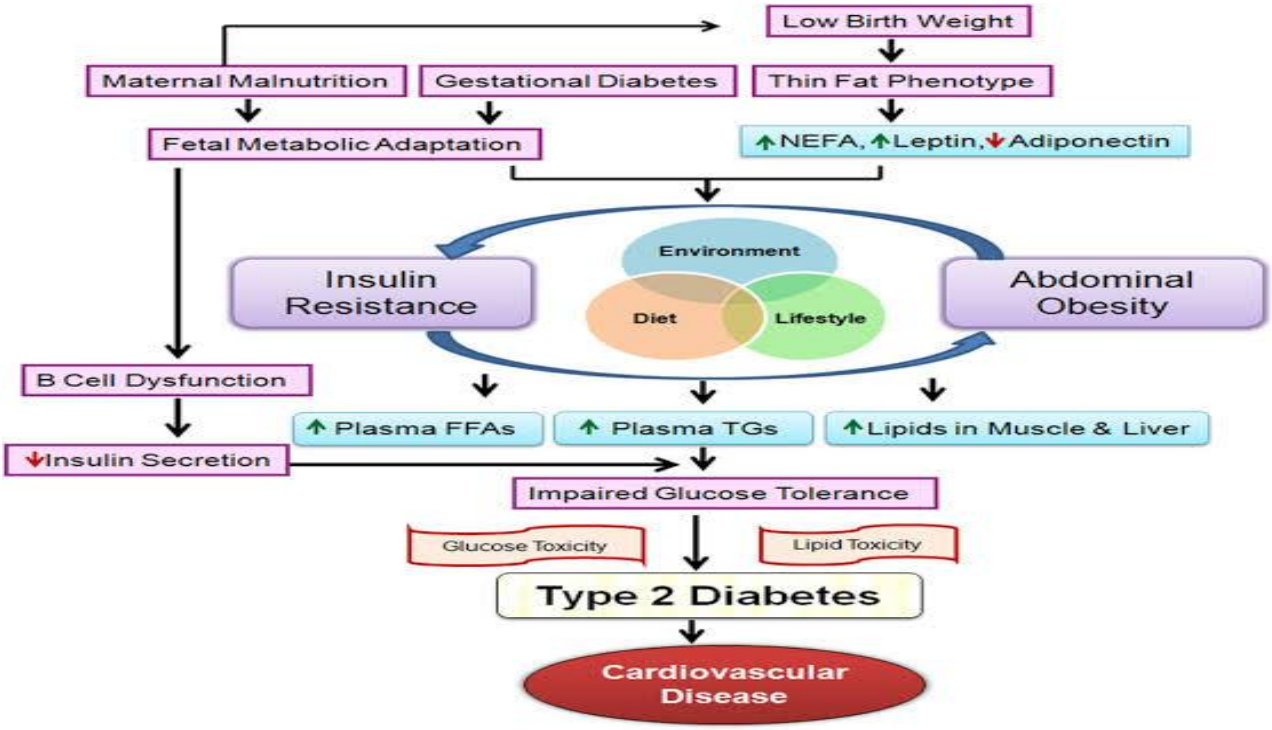

Figure 2.1: Biochemistry of the causes and effects of Diabetes

Source: Gagnum *et al*., 2017

2.4 Diabetes Causes

While the causes of diabetes vary from person to person, obesity, an unhealthy diet, alcohol consumption, and an inactive lifestyle are some of the most common factors that can lead to diabetes. More often than not, age also plays an important role. Diabetes can also be passed down through families.

Diabetes can also be caused by:

having high blood cholesterol.

Having a problem with high blood pressure.

Being diagnosed with polycystic ovary syndrome (PCOS).

Diabetes develops when the pancreas fails to release the insulin hormone, which helps your body retain sugar from food. It can occur as a result of a lack of insulin production, no production by the pancreas, or the body's insulin resistance (Gagnum *et al*., 2017).

When there is a lack of insulin hormone, the retained blood glucose from food does not enter the cells and accumulates in the bloodstream. This results in high blood sugar levels.

2.5 Diabetic symptoms and complications

Excessive thirst (polydipsia), excessive weight gain or loss, blurred vision, excessive hunger (polyphagia), fatigue, frequent infections, frequent urination (polyuria), muscle loss, dehydration, slow healing of sores and wounds, trembling, depression, dizziness, erectile dysfunction, and numbness are all symptoms of diabetes (Ciechanowski *et al*., 2003; Genuth *et al*., 2003). Chronic diabetic complications can result from hyperglycemia, which can cause glucotoxicity, which can harm various cells in the body (Wu and Yan, 2015). These negative consequences are linked to pancreatitis, diabetic retinopathy, nephropathy, hepatopathy, and neuropathy (Matsuda and Shimomura, 2013). Other diabetes complications include stroke, coronary and peripheral arterial disease (Brownlee, 2001).

Eugenol (4-allyl-1-hydroxy-2-methoxybenzene) is a well-known natural product with a pleasant scent and spicy taste found in many angiosperm plants. Eugenol has an aromatic flavor, so it is classified as an aromatic phytochemical. Eugenol is a phenylpropane that, like anethol, estragole, and cinnamaldehyde, is biosynthesized via the shikimate pathway. Eugenol is extracted from a variety of aromatic plants and used in perfumery to create spicy, clove-like, and oriental-style fragrances. Eugenol is slightly soluble in water, which is required for all medicinal applications. It is used in food as a flavoring agent. Eugenol like all phenols, is an antiseptic; it is used as a disinfectant in mouthwash. It is used in dentistry due to its antiseptic and analgesic properties;

when mixed with zinc oxide, it forms cement for temporary tooth fillings. Additionally, eugenol and methyl eugenol are used as insect attractants.

Eugenol isomers, such as isoeugenol and methyl eugenol, are also important in the evolution of organic, medicinal, natural products, and carbohydrate chemistry. Isoeugenol has been widely used as a key molecule in the development of organic synthetic methodologies; similarly, methyl eugenol is used as a flavoring ingredient in many food products, as a fragrance ingredient in perfumes, toiletries, and detergents, and as an insect attractant in some pesticide formulations. As a result, Eugenol is widely available in the marketplace.

2.6 STREPTOZOTOCIN

Streptozotocin (INN, USP) (STZ) is a naturally occurring alkylating antineoplastic agent that is especially toxic to insulin-producing beta cells in the pancreas in mammals. It is used in medicine to treat certain cancers of the islets of Langerhans and in medical research to create an animal model for hyperglycemia, Alzheimer's, type 2 diabetes, or type 1 diabetes with multiple low doses.

The Food and Drug Administration (FDA) has approved streptozotocin for the treatment of metastatic pancreatic islet cell cancer. Because it carries a high risk of toxicity and only rarely cures cancer, it is generally reserved for patients whose cancer cannot be removed surgically. Streptozotocin can reduce tumor size and symptoms in these patients (especially hypoglycemia due to excessive insulin secretion by insulinomas). A typical dose is 500 mg/m2/day administered intravenously over 5 days and repeated every 4-6 weeks.

Streptozotocin has long been used in scientific research to induce insulitis and diabetes in experimental animals due to its high toxicity to beta cells. Streptozotocin has also been used to model Alzheimer's disease in mice by causing memory loss.

2.7 MECHANISM

A glucosamine-nitrosourea molecule is streptozotocin. It is hazardous to cells through damaging DNA, similar to other alkylating chemicals in the nitrosourea class, however additional

mechanisms may possibly be at play. The activation of PARP that DNA damage causes is probably more crucial for the development of diabetes than the DNA damage itself. The glucose transport protein GLUT2 is able to carry streptozotocin into the cell because it resembles glucose enough to do so, while the other glucose transporters are unable to do so. This explains why beta cells, which have very high amounts of GLUT2, are relatively toxic to it.

2.8 *Gongronema latifolium*

Gongronema latifolium is a tropical rainforest plant that is primarily used as a spice and vegetable in traditional folk medicine. It is also known as "utazi" and "arokeke" in the South Western and South Eastern regions of Nigeria (Ugochukwu and Babady, 2002; Ugochukwu *et al*., 2013). According to reports from different writers, it also includes pregnanes, saponins, and essential oils (Schneider *et al*., 1993; Morebise and Fafunso, 1998; Morebise *et al*., 2012). Aqueous and ethanolic *G. latifolium* extracts were found to have hypoglycemic, hypolipidemic, and antioxidative properties (Ugochukwu and Babady, 2013; Ugochukwu *et al*., 2013; Ogundipe *et al*., 2013), while (Morebise *et al*., 2012) demonstrated that it has anti-inflammatory properties. These studies make little effort to look into the plant's potential nutritional and food processing/preservation values because they are primarily concerned with its medicinal properties.

A modern trend is to assess the nutritional value and chemical makeup of tropical plants, many of which have medicinal properties. In 1995, Aletor and Adeogun published a paper on the nutritional value of 17 green vegetables grown in Nigeria. Chromolaena odorata leaves' chemical make-up and nutritional value were discussed in an article by Apori *et al*,(2010). Acorus gramineus, aff. Angelica, Dendranthema indicum, Eupatorium lindleyana, Sedum aff. sarmentosum, and Sedum aff. Spectabile were all mentioned in Corlett *et al.*(2012) .'s report. While (Eleyinmi *et al*. 2006) reported on the chemical makeup of Garcinia kola seed and hull, (Ahamefule *et al*., 2006) reported on the nutritional value of Napoleona vogelii and Grewia pubescens. A deeper analysis of widely accessible but underutilized tropical medicinal plants like *G. latifolium* is required in light of the need to diversify the raw material base of the agro-allied industry and the current trend away from the use of synthetic chemicals in food processing. There is little information on *G. latifolium*'s potential uses in food. Therefore, it is crucial that

additional research be done to determine its nutritional value and evaluate its potential usage as a food or feed supplement. The study's nutrient findings would support attempts to encourage greater usage of the plant as part of a larger initiative to enlighten local residents about the nutritional advantages of the numerous wild plants that are present in their surroundings.

2.9 Description

G. latifolium, also known as utazi, is a climbing shrub with broad, heart-shaped leaves. When eaten fresh, the leaves have a distinctively sharp, bitter, and slightly sweet flavor. The stems are soft or hairy and produce exudates or milky latex.

Figure 2.2: Leaves of *G. latifolium*

Source: Ojo *et al*., 2014.

Gongronema latifolium is a plant that is used for a variety of ethnomedical and nutritional purposes in various tropical African groups. *Gongronema latifolium* Benth 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 of the plant extracts in the areas of herbal formulations, food preservation, alcoholic fermentation and beer production, drug discovery and allelopathy are also highlighted.

2.10 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 are known to have a bitter taste, form stable foams in aqueous solutions, interact with cholesterol, bile acids and other 3-β-hydroxy steroids to form mixed micelles, and to exhibit some cytotoxicity. Apart from *G. latifolium,* many other medicinal plants are also known to be rich in the saponins. Examples are Panax ginseng, Liquorice, Bupleurum falcatum and Vernonia amygdalina. Flavonoids are widely distributed and form major colouring components of plants. They are a large group of phenolic compounds and are responsible for a variety of pharmacological activities. They exist as aglycones, glycosides and methylated derivatives and can be further divided into different groups like the flavones, flavonols and flavanones. Some plant flavonoids have been shown to exhibit protective effects against infectious, cardiovascular, carcinogenic and age-related diseases.

2.11 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, 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%. They also reported that diabetic induction caused significant (P=0.05) increases in total cholesterol (TC) and LDL cholesterol (54.42% and 55.0%, respectively), compared with the normal control (NC). Treatments with the extracts significantly decreased these by 58.70% (TC) and 71.70% (LDL),

respectively. The levels of AST and ALT enzymes were significantly lowered in the extract-treated animals compared with the diabetic control. Edet et al., also reported their findings on allozan-induced diabetic rats treated with *G. latifolium* leaf extracts. In their 2009 report, the extracts lowered the serum activities of the following enzymes: creatine kinase (CK), CKMB isoform, lactate dehydrogenase, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), compared with control. They concluded that the *G. latifolium* extracts may have protective effects on both heart and skeletal muscles during cardiac and skeletal muscle diseases. In their 2011 report, *G. latifolium* leaf extracts reversed the alterations in haematological indices (WBC counts, haemoglobin count, packed cell volume) and weight loss caused by alloxan-induced diabetes in male Wistar rats. Owu *et al*., also reported the antidiabetic and antiulcerogenic effects of *G. latifolium* leaf extracts on Streptozotocin-induced diabetic rats. They also reported that the extract significantly reduced the blood glucose of the diabetic animals to levels similar to the nondiabetic control.

2.12 Reported toxicity of *Gongronema latifolium*

Through a repeated dose 90-day oral toxicity study in male and female Sprague Dawley rats, this study examined the safety profile of the ethanolic extract of *G. latifolium* (GLES) leaves do not significantly affect the hematological parameters in general or cause nephrotoxicity to occur. However, compared to the control, serum triglycerides, total cholesterol and low-density lipoprotein levels were lower and white adipose tissue paired retroperitoneal fat depots were depleted in male rats treated with GLES3 by the end of the experiment. The liver was significantly enlarged in GLES-treated rats of both sexes. Negative gender-specific alterations were observed with the highest dose. Adverse risk was evident in the female rats mainly due to marked body weight gain and cerebrum weight reduction.

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 Materials

The following material were used during the research study

Chinese cloth

Gongronemena latifolium

Aluminium foil

Wistar rats

Cages

Ceramic plates

Steel plates

Gloves

Cotton wool

Methylated spirit

Nose masks

EDTA container

Lithium heparin container

Plain container

Masking tape

Universal bottle

Test tubes racks

Lancet

250ml and 100ml beakers

Blade and scissors

Syringes (2ml and 5ml)

Insulin syringe

Pipette

Mortar and pestle

Spatula

3.2 APPARATUS

Sepearting funnel

Glass jar

Glass rod

Measuring cylinder

Dissecting set

Test tubes

Micro-centrifuge tubes

Beakers

Cuvettes

3.3 EQUIPMENT / INSTRUMENTS

Glucometer	Sigma, Germany
pH meter	Sigma, Germany
Retort stand	Sigma, Germany
Pipette (micropette)	Sigma, Germany
Weighing balance	Sigma, Germany
Oral gavage	Sigma, Germany
Spectrophotometer	Sigma, Germany
Centrifuge	Sigma, Germany

3.4 CHEMICALS AND REAGENTS USED

The following chemicals and reagents were used during the research study:

Ethyl acetate	Sigma, Germany
Sodium citrate	Sigma, Germany
Citric acid	Sigma, Germany
Ethanol	Sigma, Germany
Potassium hydroxide	Sigma, Germany
Distilled water	Sigma, Germany
Picric acid	Sigma, Germany
Chloroform	Sigma, Germany
Cholesterol reagent kit	Sigma, Germany
Triglycerides reagent kit	Sigma, Germany

Total protein reagent kit	Sigma, Germany
HDL-cholesterol reagent kit	Sigma, Germany

3.5 METHODOLOGY

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

3.5.1 Plant collection preparation

Stem bark of *Gongronemena latifolium* were obtained from Ogun State, Nigeria and identified by Dr. H.A Akinnibosun (Taxonomist) at the Department of Plant Biology and Biotechnology, University of Benin. . The pulverized extract was soaked in hydroethanol with a ratio of ethanol to distilled water 80:20 (800ml of ethanol and 200ml of distilled water) in a jar. The mixture was stirred with a glass rod until it was free. It was then allowed to stand for three days, after three days the mare was separated from the crude extract by sieving using a cheese cloth. The crude extract gotten were poured into clean jars and labelled. The mare was again soaked in hydroethanol with the same ratio of ethanol to distilled water. 80:20 in a jar for another three days. This process is termed exhaustive extraction. The crude extracts was then freezed dried in a time frame of 24hours.

3.5.2 Animals

98 Male Wistar rats were purchased from pharmacy department, with weight range 120-150, kept in a clean and serene cage and left to acclimatize for one week by feeding them ad libitum with grower mash. After acclimatization, the Wistar rats weighed 150-200 and were divided and re-grouped with seven animals in each group and kept in separate cage according to their weight range. Each rat was stained on various body parts such as their tails, abdomen, feet, head and back using picric acid. This was for identification purposes.

3.5.3 Fractionation using dichloromethane (dcm)

This was done using a separation technique and the principle behind this fraction is solvent to solvent partition it was carried out via a separating funnel. Hydro methanol (containing 75% distilled water and 25% methanol) was used to dissolve crude extract *Gongronema latifolimum*.

The mixture was then passed into the fractionationating column. Dichloromethane was afterwards added to the mixture and then agitated (shaked). The fractionationating column was then clamped to a retort stand. The dichloromethane extracts and it's phytochemicals (secondary metabolites) from the mixture while separating from it. The mixture was removed gradually from the column after separation and dichloromethane was decanted. The method was repeated till a clear dichloromethane was gotten. The DCM fractions was then freezed dried.

3.5.4 Experimental design

The male Wistar rats were arranged into 14 groups with the weights of those in a group being representative of the weight range of all the rats, such that the average weight of all the groups at onset of the experimental period was 150-200g and the groups are:

GROUP	TREATMENT
1	Normal control
2	Diabetic control(STZ only)
3	Positive control(Metformin)
4	Hydroethanol extract (200mg/kg bw)
5	Hydroethanol extract (400mg/kg)

3.6 Induction of diabetes mellitus using streptozotocin

The animals were fed for nine weeks and their weight was being monitored weekly 0until they were obsessed. The streptozotocin drug was administered through intra-peritoneal route using an insulin syringe and the fasting blood glucose was checked after three days by collecting blood samples from the tail tip of the wistar rat. And a rat was said to be diabetic until the fasting blood glucose was between 170-250mg/dl and above.

3.7 Observations (symptoms and mortality)

The animals were observed for toxic symptoms such as weakness or aggressiveness. Loss of weight, discharge from the eyes and cars, food refusal, diarrhea, noisy breathing and other physiological changes including mortality (Vijoyalakshmi *et al*., 2000).

Clinical signs that were assessed before dosing, immediately and four (4) hours after dosing include: level of sedation, urine and eye color, diarrhea, uncoordinated muscle movements. excretion of worms; restlessness, changes in nature of stool, haematuria. etc. (Fielding and Metheron, 1991).

3.8 Fasting blood sugar

The Wistar rats were starved overnight and blood was obtained from the tail tip. Methylated spirit was used to clean the tail and a lancet was used to prick the tail tip, blood was placed on the glucose test strip and inserted into the glucometer device and within 15 seconds the value for the blood glucose level was displayed on the LED screen. This was done usually every three days immediately after diabetes mellitus was induced.

3.9 Euthanization and sample collection from wistar rats

After 14 days of administration of standard drug (Metformin), DCM extracts, the wistar rats were euthanized then blood samples and liver samples were harvested from each animal. The fasting blood glucose was taken and the rats were placed into a container with cotton balls soaked with chloroform and after few minutes the rats would lose consciousness. Immediately, the rats are inverted and a section of the peritoneum cavity is cut open and extended towards the thoraxic cavity of the wistar rat using a dissecting blade.

3.10 Blood collection

Blood was collected using a 5ml syringe by piercing the inferior vena cava and cardiac puncture, and put into fluoride oxalate bottles, lithium heparin bottles and EDTA bottles. Without undue pressure to either the arm or the plunger of the syringe and micro-centrifuge-tubes and labelled boldly accordingly to the label of each animal.

The blood samples were then centrifuged at 3000 rpm revolutions for 10 min to obtain plasma sample

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of Hydroethanol extract 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 hydroethanol extract 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 4.1: Effect of Hydroethanol extract 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	183.97±2.51	190.52±2.09	+6.55±0.12
Group 5	160.25±1.50	171.1±1.19	+10.85±0.25

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, Hydroethanol extract (200 mg/kg bw), Group 5, Hydroethanol extract (400 mg/kg).

4.2 Effect of Hydroethanol extract of *G. latifolium* stem bark on Fasting Blood glucose in Streptozotocin Induced Diabetic Rats

The effect of hydroethanol extract 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. When compared to the normal control, it also returned to normal in the diabetic-treated rats 14 days after the hydroethanol extract of G. latifolium stem bark was given. After the study's fourteen-day period, the diabetic control's blood glucose level remained noticeably higher (p 0.05) than that of the normal control and diabetic treatment.

Table 4.2: Effect of Hydroethanol extract 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]	**313.25±18.02[b]	**80.00±1.15[c]	**208.46±10.43[d]
Group 5	82.60±4.14[a]	**241.25±4.37[b]	**104.00±4.85[c]	**90.25±5.20[d]

Data were presented as mean SEM, with n equal to 6. Statistics show that values with different alphabets are significant (p 0.05). When compared to the control, the mean is significant (p 0.05). When compared to the diabetic control group, the ** mean is significant (p 0.05). Group 1, the healthy control group, Group 2, the diabetic control group (STZ alone), Group 3, the positive

control group (metformin), Group 4, the hydroethanol extract group (200 mg/kg bw), and Group 5, the hydroethanol extract group (400 mg/kg).

4.3 Effect of hydroethanol extract of *G. latifolium* stem bark on total protein levels in streptozotocin induced diabetic rats

The findings demonstrate that, as compared to the normal control group, rats given streptozotocin considerably raised their total protein levels. However, when compared to diabetic controls, rats given 200g and 400g of the DCM fraction of G. latifolium significantly decreased the total protein concentration in a dose-dependent manner (STZ only). Additionally, rats given 500g of metformin demonstrated a substantial (P0.05) decrease in total protein compared to diabetic control rats.

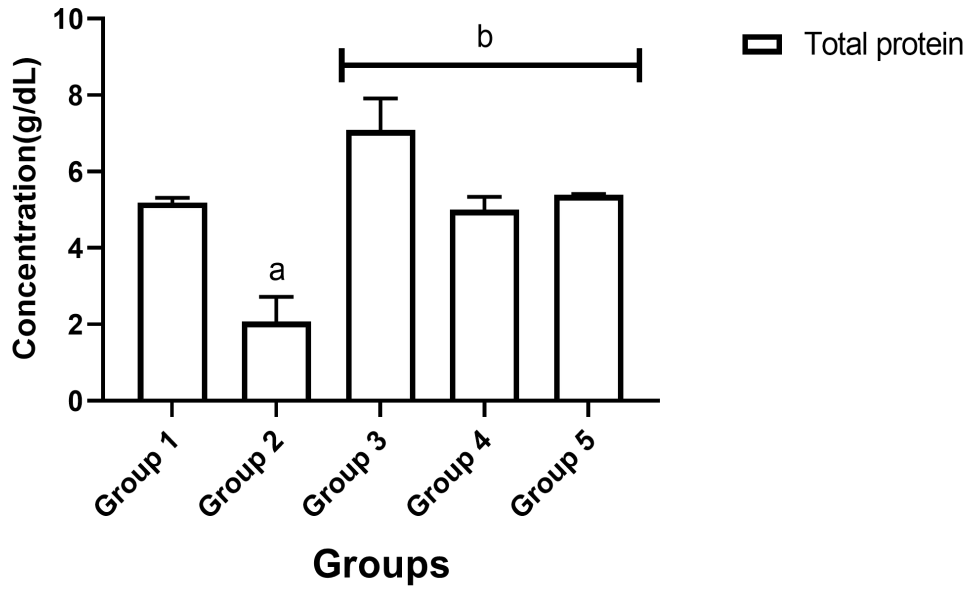

Fig 4.1: Effect of hexane fraction of *G. latifolium* stem bark on total protein levels in streptozotocin induced diabetic rats. Data were expressed as mean ± SEM, n=6. Values with different alphabet are statistically significant $p < 0.05$) Group 1, Normal control, Group 2, Diabetic control (STZ only), Group 3, Positive control (Metformin), Group 4, Hydroethanol extract (200 mg/kg bw), Group 5, Hydroethanol extract (400 mg/kg)

4.4 Effect of hydroethanol extract of *G. latifolium* stem bark on albumin levels in streptozotocin induced diabetic rats

According to the findings, albumin levels in streptozotocin-induced rats were substantially higher than those in control rats. However, when compared to diabetic controls, rats given 200g and 400g of the DCM fraction of *G. latifolium* significantly decreased Albumin levels concentration (STZ only). Furthermore, as compared to diabetic controls, rats given 500g of metformin displayed a substantial (P 0.05) decrease in albumin levels.

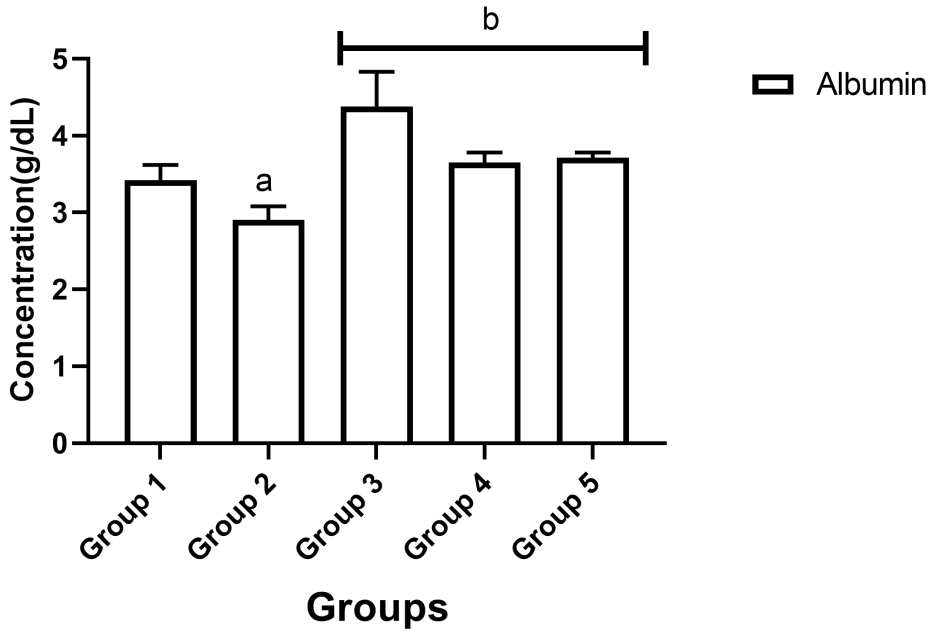

Fig 4.2: Effect of hydroethanol extract of *G. latifolium* stem bark on total protein levels in streptozotocin induced diabetic rats. Data were expressed as mean ± SEM, n=6. Values with different alphabet are statistically significant ($p < 0.05$). Group 1, Normal control, Group 2, Diabetic control (STZ only), Group 3, Positive control (Metformin), Group 4, Hydroethanol extract (200 mg/kg bw), Group 5, Hydroethanol extract (400 mg/kg)**.**

4.5 Effect of hydroethanol extract of *G. latifolium* stem bark on alanine amino transferase (ALT) activities in streptozotocin induced diabetic rats

Results indicate that, when compared to the normal control group, streptozotocin-induced rats had considerably higher levels of alanine amino transferase. But when compared to diabetic controls, rats given 200g and 400g of the DCM fraction of G. latifolium significantly decreased alanine amino transferase concentration in a dose-dependent manner (STZ only). Furthermore, when compared to diabetic controls, rats given 500g of metformin displayed a substantial (P0.05) decrease in alanine amino transferase.

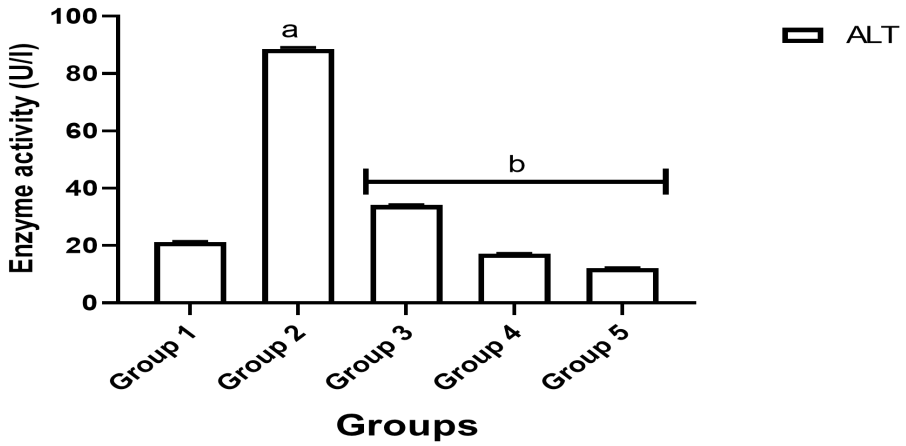

Fig 4.3: Effect of hydroethanol extract of *G. latifolium* stem bark on ALT activities in streptozotocin induced diabetic rats. Data were expressed as mean ± SEM, n=6. Values with different alphabet are statistically significant ($p < 0.05$). Group 1, Normal control, Group 2, Diabetic control (STZ only), Group 3, Positive control (Metformin), Group 4, Hydroethanol extract (200 mg/kg bw), Group 5, Hydroethanol extract (400 mg/kg)

4.6 Effect of hydroethanol extract of *G. latifolium* stem bark on aspartate amino transferase (AST) activities in streptozotocin induced diabetic rats

Aspartate amino transferase (AST) levels in streptozotocin-induced rats were considerably higher than those in the control group, according to the results. But when compared to diabetic controls, rats given 200g and 400g of the DCM fraction of G. latifolium significantly decreased aspartate amino transferase (AST) concentration in a dose-dependent manner (STZ only). Additionally, rats given 500g of metformin demonstrated a significant (P0.05) decrease in aspartate amino transferase (AST) compared to diabetic control rats.

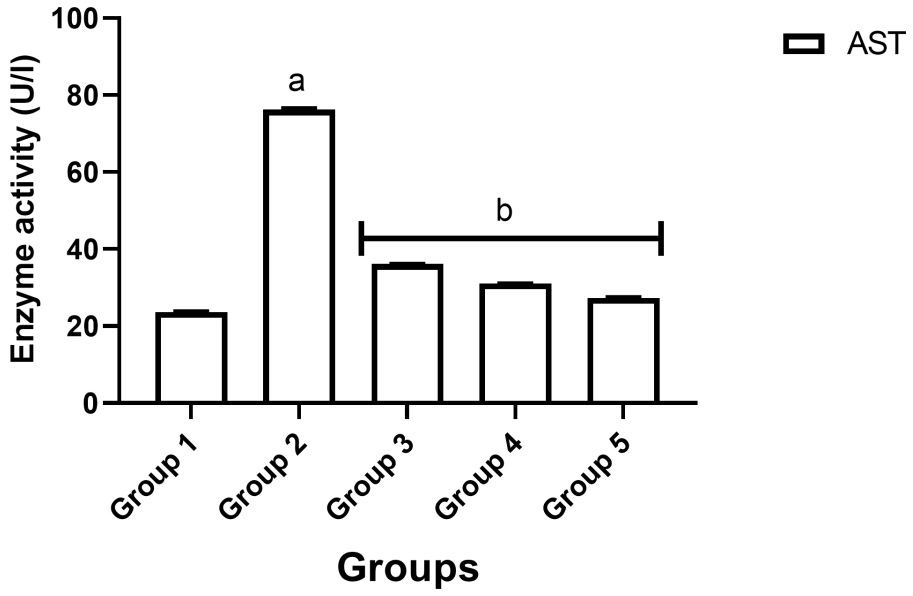

Fig 4.4: Effect of hydroethanol extract of *G. latifolium* stem bark on AST activities in streptozotocin induced diabetic rats. Data were expressed as mean ± SEM, n=6. Values with different alphabet are statistically significant ($p < 0.05$). Group 1, Normal control, Group 2, Diabetic control (STZ only), Group 3, Positive control (Metformin), Group 4, Hydroethanol extract (200 mg/kg bw), Group 5, Hydroethanol extract (400 mg/kg)

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 Discussion

The results show that streptozotocin-induced diabetes significantly reduces the weight of rats when compared to the normal control because it was noticed that the group of streptozotocin-induced diabetic rats showed weight losses that may have been caused by protein breakdown and the inability to supply amino acids for gluconeogenesis in insulin deficiency, which causes muscle wasting and tissue breakdown in diabetic rats (Mohammed et al.,2018). However, when administered a hydroethanol extract of the stem bark of *Gongronema latifolium* for 14 days, compared to the diabetic control group (Table 1), there was a steady, significant increase in body weight (4 & 5) with dose dependence. This was caused by the plant extract's high concentration of bioactive substances like saponins, flavonoids, and alkaloids, which increased the uptake of glucose by peripheral tissue and presumably reduced serum glucose levels (kim *et al.*, 2015).

Additionally, the results of fasting blood glucose (table 4.1) demonstrate a significant reduction in fasting blood sugar levels in groups 4 and 5 after administration of the hydroethanol extract. This reduction was attributed to the phytochemical composition of *G. Latifolium.*

The outcome of this study demonstrated that *G. latifolium* stem bark ethanol extracts have hepatoprotective properties (Mensah *et al*.,2008). This might be explained by the high concentrations of antioxidants called ascorbates present in *G. latifolium.* The extracts may also affect glutathione peroxidase, reducing oxidative stress, which would otherwise kill hepatocytes (Izawa *et al*.,1996).

The preservation of osmotic pressure and the binding of important chemicals, such as medications, are two functions of albumin, making it a reliable marker for the identification of liver illnesses. According to the findings, albumin levels in streptozotocin-induced rats were substantially higher than those in control rats. However, when compared to diabetic controls, rats given 200g and 400g of the DCM fraction of *G. latifolium* significantly decreased Albumin

levels concentration (STZ only). Furthermore, as compared to diabetic controls, rats given 500g of metformin displayed a substantial (P 0.05) decrease in albumin levels.

Furthermore, Hepatocytes contain the enzyme alanine transaminase (ALT), commonly known as serum glutamate pyruvate transaminase or alanine aminotransferase. When a cell is injured, its contents and those of other cells seep into the blood where they can be measured. In cases of severe liver injury brought on by xenobiotic consumption, viral hepatitis, or acetaminophen overdose, ALT rises sharply The result indicates that, when compared to the normal control group, streptozotocin-induced rats had considerably higher levels of alanine amino transferase. But when compared to diabetic controls, rats given 200g and 400g of the DCM fraction of *G. latifolium* significantly decreased alanine amino transferase concentration in a dose-dependent manner (STZ only). Furthermore, when compared to diabetic controls, rats given 500g of metformin displayed a substantial (P0.05) decrease in alanine amino transferase due to the hepatoprotective ability of *G. Latifolium.*

Similar to ALT, aspartate transaminase (AST) is an enzyme connected to liver parenchymal cells. It is also known as serum glutamate oxalate transaminase (SGOT) or aspartate aminotransferase. It is increased in cases of acute liver injury, but it is also found in red blood cells, cardiac muscle, and skeletal muscles, so it is not just a liver problem. Sometimes it helps to distinguish between the various causes of liver injury by looking at the ratio of AST to ALT.

The ALT and ALP activities in the treated animals were decreased by the *G. latifolium* extracts. This is consistent with the documents reported (Edet *et al*., 2009). According to (Nwinyi OC *et al*.,2009) the leaf extract of *G. latifolium* contains tannins, flavonoids, alkaloids, glycosides, steroids, saponins, and terpenes. There have been claims that A. dentate contains a wide variety of phytochemicals, including flavonoids. According to reports, flavonoids have antioxidant activity and are efficient superoxide anions scavengers, a phenomenon that favors hepatoprotective tendencies.

Conclusion

The result of this study indicates that the hydroethanol extract of *Gongronema latifolium* possess hepatoprotective effect with high potency due to its ability to lower liver enzymes such as ALT, AST and also Albumin and protein levels.

REFERENCES

Akpan, E.J., Okokon, J.E., Offong, E. (2012). Antidiabetic and hypolipidemic activities of ethanolic leaf extract of Melanthera scandens. *Asian Pacific Journal of Tropical Biomedicine* **2**:523-527.

Baradara, A., Madihi, Y, Merrikhi, A., Rafieian-Kopaei, M. and Nasri, H. (2013). Serum lipoprotein in diabetic patients with various renal function not yet on dialysis. *Pakistan Journal of Medical Sciences* **29**:354–357.

Cappai G, Songini M, Doria A, Cavallerano JD, Lorenzi M. (2011). Increased prevalence of proliferative retinopathy in patients with type 1 diabetes who are deficient in glucose-6-phosphate dehydrogenase. *Diabetologia* **54**:1539–1542.

Chakravarty K, Cassuto H, Reshef L, Hanson RW (2005). Factors that control the tissue-specific transcription of the gene for phosphoenolpyruvate carboxykinase-C. *Critical Reviews in Biochemistry and Molecular Biology* **40(3)**: 129–54.

Cho, N.H., Shaw, J.E., Karuranga, S., Huang, Y., da Rocha Fernandes, J.D., Ohlrogge, AW., Malanda B. (2018). IDF diabetes atlas: global estimates of diabetes prevalence for 2017 and projections for 2045. *Diabetes Research on Clinical Practices* **138**:271–281.

Garcia-Carretero, R., Vigil-Medina, L. and Mora-Jimenez, I. (2018). Cardiovascular risk assessment in prediabetic patients in a hypertensive population: The role of cystatin C. *Diabetes Metababolism Syndrome* **12(5)**:625-9.

International Expert Committee (2009). International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. *Diabetes Care* **32:**1327– 1334.

Juarez-Rojop, I.E., Diaz-Zagoya, J.C., Ble Castilo, J.L., Miranda-Osorio, P.H., CastelRodriguez, A.E., Tovilla-Zarate, C.A., Rodriguez-Hemandez, A., Angilar-Mariscal, H., Ramon-Frias, T. and Bermudez-Ocana, D.Y. (2012). Hypoglycemic effect of Carica papaya leaves in streptozotocin-induced diabetic rats. *BMC Complementary and Alternative Medicine.* **12**:236 .

Khalil, A.A., Rahman, U., Khan, M.R., Sahar, A., Mehmood, T. and Khan, M. (2017). "Essential oil eugenol: sources, extraction techniques and nutraceutical perspectives". *RSC Advances* **7(52)**:32669–32681.

Khalil, G. P., Vermaak, I. and Viljoen, A. M. (2012). "Eugenol--from the remote Maluku Islands to the international market place: a review of a remarkable and versatile molecule". *Molecules* **17(6):**6953–81.

Landon, M.B., Spong, C.Y., Thom, E., Carpenter, M.W., Ramin, S.M., Casey, B., Wapner, R.J., Varner, M.W., Rouse, D.J., Thorp, J.M., Jr Sciscione, A., Catalano, P., Harper, M., Saade, G., Lain, K.Y., Sorokin, Y., Peaceman, A.M., Tolosa, J.E. and Anderson, G.B. (2009). Eunice Kennedy Shriver National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. A multicenter, randomized trial of treatment for mild gestational diabetes. *New England Journal of Medicine* **361**:1339– 1348

Lipinski, B. (2001). Pathophysiology of oxidative stress in diabetes mellitus. *Journal of Diabetes Complication* **15(4):**203–210.

Méndez-Lucas, A., Duarte, J.A., Sunny, N.E., Satapati, S., He, T. and Fu, X. (2013). PEPCK-M expression in mouse liver potentiates, not replaces, PEPCK-C mediated gluconeogenesis. *Journal of Hepatology* **59(1)**: 105–13.

Méndez-Lucas, A., Hyroššová, P., Novellasdemunt, L., Viñals, F. and Perales, J.C. (2014). Mitochondrial phosphoenolpyruvate carboxykinase (PEPCK-M) is a pro-survival, endoplasmic reticulum (ER) stress response gene involved in tumor cell adaptation to nutrient availability. *The Journal of Biological Chemistry* **289(32)**:22090–102.

Miranda-Díaz, A.G., Pazarín-Villaseñor, L., Yanowsky-Escatell, F.G. and Andrade-Sierra, J. (2016). Oxidative Stress in Diabetic Nephropathy with Early Chronic Kidney Disease. *Journal of Diabetes Research* **2016.**

Ojo, O.A., Ajiboye, B.O., Oyinloye, B.E., Ojo, A.B. and Olarewaju, O.I. (2014). Protective effect of Irvingia gabonensis stem bark extract on cadmium-induced nephrotoxicity in rats. *Interdisciplinary Toxicology* **7(4)**:208–214.

Ojo, O.A., Ojo, A.B, Ajiboye, B.O, Oyinloye, B.E., Imiere, O.D. and Adeyonu, O. (2017). Ameliorative potentials of Blighia sapida K.D. Koenig bark against pancreatic-cell dysfunction in alloxan-induced diabetic rats. *Journal of Complementary and Integrated Medicine* **14**:20160145.

Ojo, O.A., Oyinloye, B.E., Ajiboye, BO., Ojo, A.B., Akintayo, C.O. and Okezie, B. (2014). Dichlorvos induced nephrotoxicity in rat kidney: protective effects of Alstonia boonei stem bark. *International Journal of Pharmacy* **1(7):**429–437.

Patel, D.K., Prasad, S.K., Kumar, R. and Hemalatha, S. (2012). An overview on antidiabetic medicinal plants having insulin mimetic property. *Asian Pacific Journal of Tropical Biomedicine* **2(4)**:320–330.

Pi, J., Zhang, Q., Fu, J., Woods, C.G., Hou, Y., Corkey, B.E., Collins, S., Andersen, M.E. (2010). ROS signaling, oxidative stress and NRF2 in pancreatic beta-cell function. *Toxicology and Applied Pharmacology* **244(1)**:77–83.

Prabhakar, Y.K., Janardhan, Y.E., Sreenivasulu, D., Raju, K., Kumar, K.J. and Prabhusaran, N. (2020). Ameliorative effects of Mentha aquatica on diabetic and nephroprotective potential activities in STZ-induced renal injury. *Comparative Clinical Pathology* **29:**189–199.

Prakash, O., Kumar, R., Srivastava, R., Tripathi, P. and Mishra, S. (2015). Plants explored with anti-diabetic properties: A review. *American Journal of Pharmacological Sciences* **3**:55-66.

Saeed, N., Khan, M.R. and Shabbir, M. (2012). Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts Torilis leptophylla L. *BMC Complementary and Alternative Medicine* **12(1):**221.

Taberner-Vallverdú, M., Nazir, M., Sanchez-Garces, M. Á. and Gay-Escoda, C. (2015). "Efficacy of different methods used for dry socket management: A systematic review". *Medicina Oral Patología Oral Cirugia Bucal* **20(5):**633–e639.

Uloko, A.E., Musa, B.M., Ramalan, M.A., Gezawa, I.D., Puepet, F.H., Uloko, A.T., Borodo, M.M. and Sada, K.B. (2018). Prevalence and risk factors for diabetes mellitus in Nigeria: a systematic review and meta-analysis. *Diabetes Therapy* **9**:1307–1316.

Zhang, Z., Liew, C.W., Handy, D.E., Zhang, Y., Leopold, J.A., Hu, J., Guo, L., Kulkarni, R.N., Loscalzo, J. and Stanton, R.C. (2010). High glucose inhibits glucose-6-phosphate dehydrogenase, leading to increased oxidative stress and beta-cell apoptosis. *Federation of American Societies of Experimental Biology Journal* **24**:1497–1505.