HYPOGLYCEMIC EFFECTS OF BITTER KOLA (Garcinia kola) ON ALBINO RATS

BY

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A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES IN PARTIAL FULFILMENT FOR THE AWARD OF BACHELOR OF SCIENCE, B.Sc. (HONS) SCIENCE LABORATORY TECHNOLOGY OF THE UNIVERSITY OF BENIN, BENIN CITY.

MAY, 2024.

CERTIFICATION

This is to certify that this project work was carried out by Orobosa Justina IMUDIA with matriculation number, LSC1605613 of the Department of Science Laboratory Technology, Faculty of Life Sciences, University of Benin,Benin City.

DEDICATION

I dedicate this WORK to GOD ALMIGHTY, who has blessed and sustained me through this work and has been my source of inspiration and strength.

ACKNOWLEDGEMENT

I wish to express my immense gratitude to the University of Benin and its Management for providing me with a priceless platform to commence my academic journey. The opportunity they have bestowed upon me is undeniably exceptional. My heartfelt thanks go to the Department of Science Laboratory Technology, my HOD, Prof E. O. Oshomoh and the dedicated lecturers of Physiology/Pharmacy Techniques, whose extensive knowledge and unwavering guidance have significantly influenced my academic path; for this, I am profoundly thankful.

My gratitude is indebted to my supervisor, Dr. (mrs) O. E. Obaro and co-supervisor Dr P. O. Obaro whose steadfast support, insightful contributions, and countless hours of mentoring have been invaluable throughout this project. Your dedication to my success has not gone unnoticed, and I deeply appreciate it.

To my friends; Angela, Naomi, Haruna, Success, Chidinma and Joseph who assisted me in this journey , your friendship and unwavering support have made this entire endeavor worthwhile. To the other friends I had the pleasure of meeting along the way, you all brought an element of fun and camaraderie to this journey.

Above all, I want to convey my profound gratitude to my parents, Mr. and Mrs. Imudia, my aunty Mrs Iyobosa and my uncle Professor Osewenkhae, especially my beloved mother, Mrs. Mabel Imudia. I owe everything to your love, sacrifices, and unwavering belief in me. To my dear siblings, Judith and Victory I cherish you all deeply.

Lastly, I give thanks to God Almighty, for without His divine guidance and blessings, none of this would have been possible. This journey has been a collective effort, and I am truly grateful to everyone who has been a part of it.

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ABSTRACT

Maintaining steady blood glucose levels is essential for metabolic balance and overall health. This study explores the potential of bitter kola, a rich source of bioactive compounds, to regulate blood sugar levels. Despite the global prevalence of diabetes mellitus, research on the acute and sub-acute impacts of bitter kola on blood glucose levels remains limited. The research utilizes various approaches, including testing for acute toxicity, evaluating normal blood sugar levels, and conducting anti-diabetic experiments on rats. It found that administering ethanol extracts of bitter kola at doses up to 5000 mg/kg is safe and can lower blood glucose levels in both normal and diabetic rats, indicating its potential in managing hyperglycemia and diabetes mellitus, comparable to the standard drug Glibenclamide. Moreover, the extract illustrated possible liver protection abilities and showed a positive influence on lipid levels, notably lowering cholesterol, triglycerides, and LDL levels. Crucially, it didn't negatively affect kidney tissue, indicating its safety. These results emphasize the potential of bitter kola as a natural dietary approach in managing diabetes, providing optimism for better blood sugar control and overall health.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

The blood sugar level, also known as blood sugar concentration, blood glucose level, or glycemia, is a measurement of the concentration of glucose in the blood. The body carefully controls blood glucose levels as a component of metabolic balance. Approximately four grams of dissolved glucose are maintained in the blood plasma of a 70 kg (154lb) individual at all times. Glucose can be stored as glycogen in the cells of skeletal muscle and the liver. During fasting, the body maintains stable blood glucose levels by tapping into these glycogen reserves found in the liver and skeletal muscle. The circulation of blood can move glucose from the intestines or liver to various tissues in the body. Insulin, which is produced in the pancreas, serves as the main controller of how cells take in glucose. (Wasserman, 2009)

In humans, maintaining sufficient glucose levels is essential for the proper functioning of various tissues, including the brain, which utilizes approximately 60% of the body's blood glucose when adults are fasting and at rest. Persistent elevation in blood glucose levels leads to glucose toxicity, leading to cellular dysfunction and the development of diabetic complications. Typically, glucose levels are at their lowest in the morning, prior to the first meal of the day, and they rise by a few millimoles for an hour or two after eating. Hyperglycemia refers to abnormally high sustained glycemia; hypoglycemia refers to low levels (Wasserman, 2009).

1.2 Low Blood Sugar Level (Hypoglycemia)

It denotes a blood glucose level below 70 mg/dL. Blood glucose levels might fall for a variety of causes, including skipping meals, taking diabetic medication, or exercising more than usual. Taking insulin or diabetes drugs that encourage the secretion of insulin increases the risk of hypoglycemia. A low blood glucose level can be corrected by drinking or eating something sweet. (Peiro *et al.,* 2014)

1.3 High Blood Sugar Level (Hyperglycemia)

Hyperglycemia is a condition in which there is an excess of glucose in the blood plasma. This is typically a blood sugar level greater than 11.1mmol/L (200 mg/dL), however symptoms may not appear until much higher levels, such as 13.9-16.7mmol/L (250-300 mg/dL). A patient with a constant range between 5.6 and 7mmol/L (100-126 mg/dL) is termed slightly hyperglycemic, while anyone with a range above 7 mmol/L (126 mg/dL) is declared diabetic. In individuals with diabetes, what is considered excessively high glucose levels can differ from one person to another, primarily due to variations in their renal threshold for glucose and their overall glucose tolerance. Chronic levels above 10-12 mmol/L (180-216 mg/dL) can, on average, cause obvious organ damage. (Peiro *et al.,* 2014)

1.4 Diabetes Mellitus

The terms "Diabetes" and "Mellitus" originate in Greek. "Diabetes" means "passer-by: a siphon" and "mellitus" means "sweet". Diabetes mellitus is a type of insulin resistance syndrome characterized by persistent hyperglycemia (high blood sugar levels). The ailment is referred to locally as diabetes or sugar disease. (Peiro *et al.,* 2014)

The diverse causes of this condition encompass abnormalities in carbohydrate, fat, and protein metabolism, as well as challenges related to insulin secretion, insulin function, or both. Over the long term, diabetes can lead to specific complications like retinopathy, nephropathy, and neuropathy. Furthermore, diabetes heightens the likelihood of various other health issues, including nonalcoholic fatty liver disease, cataracts, erectile dysfunction, peripheral artery and cerebrovascular diseases, heart disease, and obesity. People with diabetes face an elevated risk of contracting specific infectious diseases, such as tuberculosis. Diabetes can manifest with distinct symptoms like heightened thirst, frequent urination, vision impairments, and weight loss. Additionally, diabetic patients commonly experience genital yeast infections. Ketoacidosis or a non-ketotic hyperosmolar condition, which can result in dehydration, a coma, and, in the absence of adequate treatment, death, are the most serious clinical signs. (Zimmet *et al.,* 2001)

It has nothing to do with "Diabetes Insipidus," which is a kidney-related fluid retention condition. Diabetes mellitus is classified into four types: Type 1 Diabetes Mellitus (T1DM), Type 2 Diabetes Mellitus (T2DM), Gestational Diabetes Mellitus (GDM), and Other Specific Types (Alam *et al.,* 2017).

Around 2019, there were about 463 million individuals worldwide who had diabetes. Diabetes mellitus affects around 8.8% of adults, with Type 2 Diabetes mellitus (T2DM) accounting for roughly 90% of cases. An estimated 4.2 million deaths were attributed to diabetes mellitus in 2019. (Shaw and Cummings, 2012)

Diabetes mellitus doubles the risk of premature mortality. Type 1 diabetes mellitus (T1DM) occurs due to inadequate insulin production by pancreatic cells, while Type 2 diabetes mellitus (T2DM) begins with insulin resistance, where cells don't respond properly to insulin. Gestational Diabetes Mellitus arises in pregnant women with no previous diabetes history, resulting in elevated blood sugar levels. T1DM can be effectively controlled by administering insulin injections. T2DM treatment entails following a good diet, maintaining a healthy weight, and engaging in physical activity (Nagesh *et al.,* 2020). Diabetes is treated with a class of insulin medicines that includes insulin secretagogues (Sulphonylureas, Meglinides); insulin sensitizers (Biguanides, Thiazolidinediones); alpha-glucosidase inhibitors; and sodium-glucose co-transporter 2 inhibitors (Bhalova *et al.,* 2021).

1.5 TYPES OF DIABETES

1.5.1 Insulin Dependent Diabetes Mellitus (Type 1 Diabetes Mellitus)

This type of diabetes mellitus is often referred to as auto-immune diabetes and was formerly known as juvenile onset diabetes. It is also referred to be polygenic disorder that is ketosis-prone (Kumar *et al.,* 2020). This occurs mainly in children, and Adults and it develops before the age of 40, the disease occurs suddenly in teenagers and it can also be life-threatening. Insulin can be used to manage or treat T1DM, but there is no known cure. This condition was originally called as "Insulin-Dependent Diabetes Mellitus." T1DM's precise causes aren't known (Nagesh *et al.,* 2020). Insulin is necessary to treat those with T1DM. T1DM arises from the pancreas's incapacity to generate sufficient insulin because of the loss or destruction of beta cells. Elevated blood sugar levels can lead to both immediate and chronic complications. T1DM is a chronic metabolic condition characterized by an inability to produce insulin. Since food-derived glucose cannot reach cells, its content in the blood is raised. 10% of persons have type 1 diabetes mellitus. (Lal, 2016)

1.5.2 Non-Insulin Dependent Diabetes Mellitus (Type 2 Diabetes Mellitus)

This is also known as Adult onset Diabetes Insulin secretion defects identified in T2DM contribute to Insulin Resistance (IR). This condition was originally called as "non-insulin-dependent diabetes mellitus." It induces insulin resistance and beta cell abnormalities as a result of glucose buildup in the bloodstream. It is a chronic condition that worsens over time. A condition in which cells fail to respond to Insulin properly or reduced insulin level develops hyperglycemia and free fatty acid. T2DM affects blood vessels and the heart, as well as causing disease and death in the patient. The predominant cause usually results from both being overweight and not engaging in physical activity. Firstly, it's developed pre-diabetes. Long-term renal issues are a result of T2DM (Kumar *et al.,* 2020).

1.5.3 Gestational Diabetes Mellitus (GDM)

Those who are pregnant and have this Diabetes are classified as having GDM. It is the most prevalent kind of heritable monogenic illness. Women suffering from GDM require Insulin or other medications. Gestational diabetes ranks as the third most prevalent type, affecting pregnant women who have not previously experienced diabetes. GDM is typically identified in the later stages of pregnancy and frequently occurs in women with no prior history of diabetes mellitus. A fetus born with diabetes mellitus is at significant risk of developing obesity and T2DM in the future. GDM is divided into two classes: A1 and A2. It happens in approximately 4% of all pregnancies. Diet and exercise address ClassA1 situations. Globally, gestational diabetes is expected to rise by 18% (Gupta and Tyagi, 2017).

1.6 OTHER SPECIFIC TYPES OF DIABETES MELLITUS (MONOGENIC TYPES)

It is also caused by genetic anomalies in insulin hormone internal secretion. Mutations cause it in 1 to 5% of those who have suffered from it. This includes pancreatic disorders, specific surgeries, genetic problems in beta cells, cancer therapies, medicines, and infections, among other things. Some medications are used or combined with HIV/AIDS treatment or organ transplantation. It comprises the MODY, LADA, and Endocrinopathies subtypes. (Adnette *et al.,* 2019).

1.6.1 Mody

Insulin may or may not be required. MODY is an abbreviation for Maturity-Onset Diabetes of the Young. MODY diabetes is inherited diabetes mellitus caused by a genetic mutation in an autosomal dominant gene that alters insulin secretion or production, and it is not an insulin-dependent diabetes. Individual diagnosis is usually made in children under the age of 25 who have hereditary variables. MODY is caused by the HNF1-Alpha (Hepatocyte Nuclear Factor) gene in around 70% of cases. It is linked to a genetic abnormality in - cells. Hyperglycemia occurs at a young age in this kind. They are clinically similar to type 2 diabetes. (Jones and Hattersley, 2010)

1.6.2 Lada

LADA stands for Latent Autoimmune Diabetes in Adults in its full form. Insulin is not necessary for months to a year after diagnosis. They cannot produce insulin, and it is clinically comparable to T1DM. LADA arises when pancreas cells stop producing insulin. LADA is found in young individuals in their twenties and, due to age, might be misdiagnosed with type 2 diabetes mellitus (Chaudhary and Tyagi, 2018).

1.6.3 Endocrinopathies

Several hormones can influence insulin action or decrease insulin action. Impaired fasting blood glucose is characterized by an FBG greater than 100mg/dl but less than 126 mg/dl. It may include polycystic ovarian syndrome, pancreatic cancer or tumors, and other hormonal disruptions in insulin production. (Chaudhary and Tyagi, 2018)

1.7 PATHOPHYSIOLOGY OF DIABETES

T1DM is an autoimmune disorder characterized by the death of pancreatic β-cells by T-cells, resulting in insulin insufficiency and, eventually, hyperglycemia. Though not fully understood, the pathophysiology of this immunity has been discovered to be impacted by both genetic and environmental variables. In most situations, the rate of development of this pancreatic -cell-specific autoimmunity and the illness itself is quick, as in infants and adolescents (juvenile onset), or it may be slow, as in adults (late onset). (Kahaly and Hansen, 2016)

Type 2 diabetes mellitus (T2DM) is characterized by two insulin-related abnormalities: insulin resistance and β-cell dysfunction. Insulin resistance occurs due to disruptions in various cellular pathways, leading to a decreased response or sensitivity to insulin in peripheral tissues such as muscles, liver, and fat tissue. Initially, this reduced insulin sensitivity causes β-cells to compensate by working harder to regulate normal blood glucose levels through increased insulin secretion (hyperfunction). This excess insulin prevents high blood sugar (hyperglycemia). However, as time passes, the heightened insulin secretion cannot fully compensate for the ongoing decline in insulin sensitivity. Furthermore, the function of β-cells deteriorates, causing an insulin shortage. Consequently, neither normal blood glucose levels nor high blood sugar levels can be sustained.

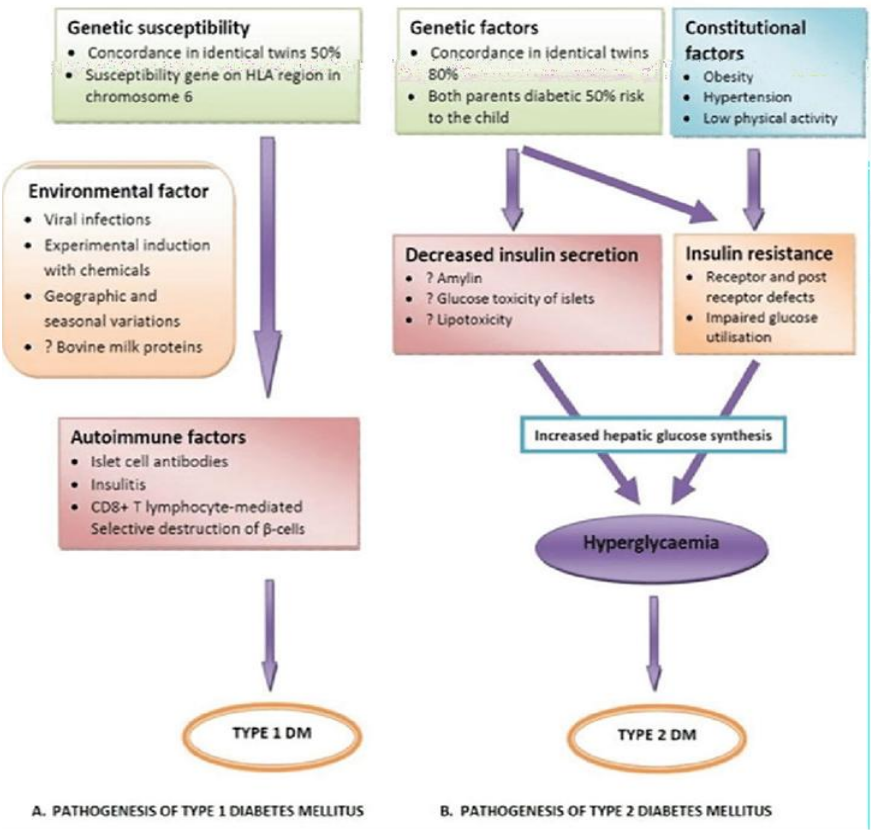

Figure 1.1 Pathophysiology of type 1 and type 2 diabetes.

(Source: Parveen *et al.,* 2017)

1.8 SIGNS AND SYMPTOMS OF DIABETES

Signs	Symptoms
Extreme Hunger (Polyphagia)	Dry Skin and Mouth
Excessive Thirst (Polydipsia)	Foot Pain
Frequent Urination (Polyuria)	Yeast Infection
Slow Wound Healing	Genital And Skin Infection
Acanthosis Nigraicans	Fatigue
Weight Loss	Nausea
Dehydration	Pain In Stomach
Headache	Vomiting
Flushed Face	Blurry Vision

Table 1.1; Signs and symptoms of diabetes mellitus

1.9 MANAGEMENT OF DIABETES

1.9.1 Diet

Doctors recommend a high-fiber, low-fat diet rich in fruits, vegetables, and whole grains. Foods containing clarified sugar should be avoided. Because alcohol contains a lot of sugar, alcohol consumption (Alcoholism) should be decreased. (Gregery *et al.*, 2013)

1.9.2 Exercise

Exercise has an excellent effect on physical, mental, and social wellbeing. Engaging in physical activity helps lower blood sugar levels. Consistent exercise also contributes to managing a desirable body weight and keeping high blood pressure and cholesterol levels in check. As a result, the risk of linked health issues such as cardiovascular disease is reduced. Sitting still (being sedentary) for extended periods of time is a risk factor for T2DM. (Gregery *et al*., 2013)

1.9.3 Insulin Therapy

T1DM patients require insulin therapy. The purpose of insulin therapy is to keep blood sugar levels stable or under control. It is given subcutaneously with a syringe, insulin pen, or insulin pump. Insulin therapy is the most critical aspect of T1DM and, in some cases, T2DM. (Gregery *et al.,* 2013).

1.9.4 Anti-diabetic drugs

Antidiabetic drugs are medications designed to manage and control blood sugar levels in individuals with diabetes mellitus. They play a role in controlling glucose metabolism and can function through various mechanisms, such as boosting insulin production, improving insulin sensitivity, or delaying carbohydrate absorption. Examples include metformin and sulfonylureas like glibenclamide (Gregery *et al.,* 2013).

1.10 Glibenclamide

Glibenclamide, often known as glyburide, is a second-generation sulfonylurea drug that is the most commonly prescribed oral hypoglycemic medication. Chemically, it is a sulfonylurea molecule, 5-chloro-N-[2-[4-cyclohexyl carbamoyl sulfamoyl) phenyl] ethyl]-2-methoxy benzamide, which operates as either pancreatic or extra pancreatic, increasing insulin release from beta cells in the pancreatic islets (Nathan *et al.,* 2009).

1.11 Mechanism of action

In hyperglycemic situations, glibenclamide lowers blood glucose by boosting insulin synthesis from the pancreas's existing beta cells. In addition to this direct action, it exhibits other pancreatic effects. This drug binds to the sulfonylurea receptor 1 (SUR 1), a regulatory subunit of the pancreatic beta cells' ATP-sensitive potassium channels (KATP). This inhibition results in the depolarization of the cell membrane and the activation of voltage-dependent calcium channels, leading to an increase in the intracellular calcium concentration within beta cells. This, in turn, amplifies the release of insulin. Thus, the single medication glibenclamide increases insulin secretion while decreasing hepatic glucose synthesis (Sharma and Kar, 2014)

1.12 AIM OF STUDY

This study was aimed to evaluate the acute and sub-acute hypoglycemic effect of (Garcinia kola) bitter kola

1.13 OBJECTIVES OF STUDY

The objectives of this study are to;

- determination of the acute hypoglycemic effect of bitter kola on normoglycemic wistar rats.
- determination of the sub-acute hypoglycemic effect of bitter kola in normoglycemic wistar rats.
- determination the anti-diabetic effect of bitter kola on streptozocin induced diabetic wistar.

CHAPTER TWO

LITERATURE REVIEW

2.1 BIOLOGY OF THE PLANT

Garcinia kola is a member of the Clusiaceae family and is sometimes referred to as African miracle nut and bitter kola. This dicotyledonous plant grows in swamps and damp rain forests. Known for its many uses in agroforestry, Garcinia kola is considered one of Nigeria's most significant medicinal plants. Although the nuts were originally chewed as a masticatory material to increase salivary flow, they are now commonly eaten as a snack in West and Central Africa (Leakey, 2001).

Bitter kola is a multipurpose agroforestry tree species native to Africa's Western and Central regions (Agyili *et al.,* 2020). The species' hotspot includes a belt of countries from Ghana in the East to Gabon in the South-West. The tree is dioecious, occurring in lowland tropical forests and growing up to 40 m in height (Smith *et al.,* 2022). It is most valued for its medicinal properties of its seeds, bark, and leaves (Tshibangu *et al*., 2016). These plant parts are generally used to either cure or relieve the symptoms of several common ailments, including gastrointestinal issues, headaches, respiratory issues, liver disorders, and gonorrhoea, among other (Oluwatosin *et al.,* 2014).

Even when a lot of nuts are consumed, garcinia kola is thought to cleanse the digestive tract without causing negative side effects like stomach issues (Onochie, 1960). For generations, folk medicine has utilized the plant's fruit, seeds (bitter kola nuts), and bark to cure a variety of illnesses, from fever to cough.

2.2 TAXONOMIC CLASSIFICATION OF THE PLANT

Kingdom: Plantae

Phylum: Tracheophytes

Class: Magnoliopsida

Subclass: Eudicots

Order: Malpighiales

Family: Clusiaceae

Genus: Garcinia

Species: Garcinia kola. (Yakubu *et al.,* 2014)

Plate 2.1 Picture of *Garcinia kola*

2.3 DISTRIBUTION AND ECOLOGY

Garcinia kola grows naturally in a range that stretches from the Congo to Sierra Leone (Constant *et al.,* 2013). Cameroon and Nigeria are typically thought to be the species' main hotspots. Despite the fact that the species is known to be found in coastal areas and lowland plains up to 300 m above sea level, trees can be successfully grown even in hilly regions around 750 m above sea level (Agyili, 2017). Köppen-Geiger classifies the climate zones in which G. kola often occurs as "tropical rainforest climate, tropical monsoon climate, or tropical savannah climate" (Kottek *et al.,* 2006). The average annual temperature in such regions is between 21°C and 31°C, while the mean annual precipitation is between 1000 and 3000 mm. A comparatively high air humidity of roughly 75% goes hand in hand with this (Babalola *et al.,* 2010).

2.4 CHEMICAL COMPOSITION OF BITTER KOLA

7.2%–92.7% for moisture; 0.33%–5.9% for ash; 0.58%–7.8% for crude protein; 0.19%–14.5% for crude fat; 1.23%–20.51% for crude fiber; and 10.85%–91.35% for NFE. The majority of research concur that the seeds should have a reasonably high moisture content—roughly 70%—which is essential for maintaining the kernels. Nitrogen-free extracts (NFE), another name for carbohydrates, make up the majority of the seed's proximate composition, which can vary from 65% to 100%. However, the ash content—which is only 1.5%—is extremely low and comes from burning the entire sample to inorganic substituents. Crude protein has an average value of 3.5%, crude fat has an average value of 6.2%, and crude fiber has an average value of 9.4% (Onyekwelu *et al.,* 2015).

2.5 ACTIVE CONSTITUENTS OF BITTER KOLA

Flavonoids are the phytochemicals that are most prevalent in Garcinia kola seeds. Many writers have also confirmed the presence of alkaloids, glycosides, tannins, phenols, and saponins. Despite the detection of anti-nutrients such phytate and oxalate, the seeds are safe to eat, and no negative overdose cases have been reported to date (Konziase, 2015). Low molecular weight chemicals called flavonoids are regarded as natural antioxidants because of their capacity to scavenge free radicals and convert them into innocuous molecules. They also have an effect on the body's immune cell activation in different ways. The chemicals function as anti-tumor (benign, melanoma) agents (Altemimi *et al.,* 2017) and are helpful in shielding the central nervous system from oxidative and excitotoxic stressors (Nworu *et al.,* 2008). The kolaviron biflavonoid complex (KV) is one of the parts of Garcinia kola seeds that has been investigated and discussed the most. The biflavanones GB1, GB2, and kolaflavanone are also included in this complex (Tchimene *et al.,* 2015). Tannins, secondary metabolites of Garcinia kola kernels that are recognized for their natural ability to treat intestinal illnesses like diarrhea and dysentery, are the source of the kernels' characteristic astringent flavor. In addition to their antimicrobial qualities, tannins have also been linked to a significant chance of preventing cancer (Usunomena *et al.,* 2012), and when combined with phlobatannins, they demonstrate the ability to heal wounds (Kagbo, 2010). Lastly, Garcinia kola preparations included steroidal substances as well as cardiac glycosides. This is consistent with the plant's historic use in treating chest pain and heart infections, as well as the fact that men chew the seeds for aphrodisiac effects (Usunomena *et al.,*2012).

2.6 PHARMACOLOGICAL BENEFITS OF BITTER KOLA

2.6.1 Antihypertension:

Hypertension, well-known as high blood pressure, is considered by persistently excessive blood pressure in the arteries (Baharvand *et al.,* 2016). High blood pressure can damage arteries supplying blood to the kidneys, heart, brain, and eyes (Baharvand *et al.,* 2016). The blood pressure of rats fed Garcinia kola enriched meals dropped significantly by the third week. Finally, Garcinia kola contains a vasoactive component that can reduce blood pressure. Traditional medicinal practitioners have always advocated for using Garcinia kola parts to treat hypertension.

2.6.2 Antidiabetic:

The most common symptom of diabetes mellitus is hyperglycemia, a metabolic disorder. The kidneys, heart, nerves, and blood arteries are all affected by its chronic stage (Abdulraham, 2021). Globally, 463 million individuals suffer from diabetes, and by 2030, that figure is predicted to increase to 578 million (Abdulraham, 2021). In both normal and alloxan diabetic rabbits, kolaviron linked bioflavonoids significantly reduced hypoglycemia symptoms. There was no significant difference in body weight, long-term HDL levels, or single-dose glucose levels compared to the controls. Nonetheless, the treated rats had considerably lower glucose levels than the controls, and the treated group's LDL levels were 66% lower. Blood glucose levels were 49.70% lower in the group treated with ethanolic seeds extract than in the positive control group (45.03%). According to the investigation's findings, the seed may be utilized to manage diabetes and treat ailments (Omodamiro *et al.,* 2020)

2.6.3 Antipneumonia:

Pneumonia is an infectious, inflammatory lung illness that can be either acute or chronic that damages the lung's mucosal surfaces (Calista *et al.,* 2021). The condition is caused by viruses, bacteria, and fungi. Anti-Klebsiella pneumonia activity increased in response to a decrease in kolaviron levels. Bitter kola's antimicrobial qualities allow it to treat pneumonia.

2.6.4 Antiviral:

The results of this study indicate that the extract has a clear and promising capacity to treat a patient's ocular symptoms and signs right away. This might revolutionize the way these viral infections are treated, as there is currently no specific antiviral drug available (Adefule et al., 2004). This study shows that Garcinia kola works well against viral infections and in resource-poor areas.

2.6.5 Anti-Inflammatory:

The body's normal reaction to injury or external irritability is inflammation. Humanity has been aware of inflammation, a painful condition, since the beginning of time. Humans have been searching for methods to control and lessen inflammation since the beginning of time, and one such method is the use of plants (Mahmoud and Abba, 2021). The results of the investigation were influenced by the addition of substances with anti-inflammatory properties (Adedapo *et al.,* 2015). Under circumstances where there is cellular proliferation and inflammatory responses, it might be advantageous (Adedapo *et al.,* 2016).

2.6.6 Antifungal:

The use of plant extracts as a source for the development of novel antifungal drugs has a long history. Plant-based medications have significantly improved human health and well-being. The data shows that the extracts also have antifungal activity against Aspergillus niger. The compound has substantial antifungal activity when compared to the standard antibiotics used in the study, with values comparable to ketoconazole (Babandoko *et al.,* 2012). The seed extract also exhibits high activity against Candida albicans and Aspergillus flavus in a fungistatic manner. The findings of this study suggest that the extract may contain compounds that could be effective in combating microbial diseases. (Jude *et al*., 2020).

2.6.7 Analgesic:

Managing acute and chronic pain has become a serious concern, especially among the elderly. Pain is a nonspecific symptom of many diseases that can lead to unpleasant emotional and sensory experiences. The findings of the study show that the chemical has dose-dependent antinociceptive properties against acetic acid-induced abdominal constriction in mice. At all doses, the number of writhes was reduced compared to control animals. The seed of bitter kola has been shown to have analgesic properties (Iniaghe and Onyemaonyeoru, 2015). The studies examined in the following study found that the extract from bitter kola had a significant analgesic effect

2.6.8 Obesity management:

Obesity is a complex health condition that is considered a chronic disease and can have detrimental effects on the human body (Winters *et al.,* 2006). Obesity increases the risk of diabetes, hypertension, heart disease, and other serious health conditions (Hossain *et al.,* 2007). The number of people who are obese around the world is increasing at an alarming rate (Seidell, 2000). According to current estimates, there are over 300 million people in the world who are considered obese (Seidell, 2000).

The results showed a significant increase in the number of RBCs in both tested animals, as well as a decrease in their weight. There was a decrease in very low-density lipoproteins in the plasma in a dose-dependent manner, while chylomicrons increased in a dose-dependent manner. The decrease in high-density lipoproteins and increase in low-density lipoproteins contribute to cardiovascular disease.

2.6.9 Antiglaucoma:

Glaucoma is the leading cause of permanent blindness worldwide (Jeduah *et al.,* 2020). The most common type, primary open-angle glaucoma (POAG), affects over 60 million people worldwide. In Africa, glaucoma is responsible for 15% of blindness (Jeduah *et al.,* 2020). After oral consumption, the intraocular pressure of healthy young people was lowered by 21%, which may be beneficial for patients with POAG or ocular hypertension in low-income settings.

2.6.10 Healing of Liver Injury:

The liver is a vital organ in our body responsible for most metabolic and secretory functions. This makes the liver a sensitive target for drugs that modify or alter metabolic processes (Bhoumik *et al.,* 2014). In clinical practice, liver injury can be broadly classified as acute or chronic depending on the duration or persistence of the injury (Mahi and Goves, 2008).

2.6.11 Cytotoxicity:

Many plant-derived chemicals have been shown to possess a wide range of therapeutic properties, including antibacterial, anticancer, and other beneficial biological effects (Mohamidi *et al.,* 2017). The parts of medicinal plants are believed to harbor novel compounds with therapeutic potential that could be effective in treating a variety of diseases, and may offer advantages over synthetic drugs (Hamad *et al.,* 2014). Studies have shown that medicinal plants contain a wide array of compounds with positive biological effects (Abdulraham *et al.,* 2021). It is important to note that these beneficial components are only beneficial if they are confirmed to be non-toxic or minimally toxic. Several studies have been conducted to determine the toxicity of Garcinia kola parts both in vivo and in vitro. It was found that increased dietary intake of Garcinia kola seeds significantly decreased the survival rate of D. melanogaster compared to control flies (Oboh *et al.,* 2018). The decreased survival rate of D. melanogaster may be linked to the bioactivity of G. kola seed components, such as saponins and glycosides, which are known to be hazardous in large doses (Kagbo and Ejegbe, 2010). The extract did not appear to have significant toxicological effects on erythrocytes, although it did tend to increase their number over time (Kagbo and Ejegbe, 2010). The results showed that both medicinal plant extracts had no significant negative effects on total protein or glutamate pyruvic transaminase at compared to the control (Farombi *et al.,* 2013). The toxicology of Garcinia kola has been found to be relatively mild, with an oral 50% fatal dose exceeding 5000 mg/kg (Farombi *et al*., 2013)."

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection of Research Materials

The *Garcinia* kola (EGC) directly purchased from Oluku Market Ovia North East local Government Area of Edo State, Benin City Nigeria. The plantain was peeled and the peel was cleaned and air dried at room temperature for 2 weeks and transferred to the oven for 24 hours after which the *Garcinia kola* was weighted and pulverised into powdery form and stored in air tight container.

3.2 Extraction of *Garcinia kola*

The powdered *Garcinia kola* was extracted by method of cold maceration. 1000 g of the powdered *Garcinia kola* (EGC**)** was soaked in 2.5 litters of Ethanol and scooped vigorously in an air tight jar and wrapped with black cloth which was kept in a cupboard and monitored regularly and kept for 73 hours. After 73 hours, cheese cloth was used to separate the plant fiber from the concentrate and transferred to the oven for 48 hours at a controlled temperature of 40^0c.The dried sample was weighed and the yield was calculated.

3.3 Acute Toxicity Study

Acute toxicity study was carried out by methods of (OECD, 2018) Organization of economic co-operation development guidelines. Three (3) male mice and 3 female mice each were administered 5000 mg/kg of the Ethanol extract of *Garcinia kola*(EGC) and observed for 24 hours for possible signs of toxicity, mortality or morbidity.

3.4 Anti-diabetic Study

Ethanol extract of *Garcinia kola* (EGC) was administered to normoglycemic and diabetic induced Wistar rats using streptozotocin. The blood sugar level of normoglycemic rats were evaluated blood sugar level (Bsl) and blood sugar level (Bsl) of diabetic rats on streptozotocin induced diabetes in rats treated with three doses of aqueous sawasop smoothy for 0- 73 hours and 20 days at the doses of 150, 500 and 5000 mg/kg.

Thirty (30) days oral administration of EGC at the doses of 500 and 250 mg/kg to streptozotocin induced diabetic rats after which the animals were anesthetized and blood was collected from the abdominal aorta. Collected blood samples were screened for the following parameters: Alkaline phosphates (ALP), Aspartate amino transferase (AST), Alanine amino transferase (ALT) and total protein (TP).

3.5 Experimental animals

Albino rats weighing 200g to 250g were purchased from the Department of Anatomy Animal House Facility University of Benin.

3.6 Blood glucometer check

Accu-check active glucometer and visual blood glucose test stripes, products of Roche diagnostic Gmbh, D-68298 Mannheim, Germany were used for the fasting blood glucose level estimation

3.7 Drugs /chemical

Streptozoticin, Gilbenclamide, ethanol, rat restrainer, pelletized feed, cotton wool, methylated spirit, pocket PH meter, glass slide, cover slips, microscope, centrifuge, hand cloves, plastic cages

3.8 Statistical Analysis

The results from the studies were expressed as mean ± SEM. Statistical analysis were carried out using graph pad prism 6 version software (UK). Comparisms between the control and treated groups were analysed using one-way ANOVA and, Dunnett's multiple comparisms test. * = $P < 0.05$, was regarded as indicating significant difference.

CHAPTER FOUR

RESULT

Table 4.1: Effect of ethanol extract of *Garcinia kola* on liver function indices

Parameter	Control	Gilben 5 Mg/kg	D. Control Strep induced	EGC 150 Mg/kg	EGC 500 Mg/kg
ALP	65.2±0.2	60.2±1.3*	101.0±2.3	49.5±1.2[a]	49 ±0.9[a]
ALT	50.1±0.7	47±0.5	108±1.0	36.4±1.0[a]	37±2.1[a]
AST	55.0±0.9	43.6±0.1	106.4±0.2	42.0±1.5[a]	40±1.6[a]
T P	6.9±0.4	6.1±0.7	14.0±1.0	6.5±0.01[a]	27±0.02[a]

Results are expressed as mean ± SEM (n= 5) [a] = $p < 0.05$ represent significant decrease

Key: EGC = Ethanol extracts of *Garcinia kola*, D. Control =Diabetic Control, Gilben. = Gilbenclamide.

Table 4.2 : Effect of ethanol extracts of *Garcinia kola* on Lipid profile indices.

Parameter	Control	D. Control	EGC 150 mg/kg	EGC 500 mg/kg
TG	63.0±0.9	108.0±2.0^{d}	49.0±1.0	48.7±0.8^{d}
HDL	64.0±0.1	190.5±0.4^{d}	47±0.6^{d}	44.5±1.7^{d}
LDL	51±1.0	180.1±0.3^{d}	24.1±1.1^{d}	25±1.3^{d}
TC	84.0±3.0	160.6± 0.9^{d}	78.0± 0.4^{d}	76.3± 1.0^{d}

Results are expressed as mean ± SEM (n= 6), significant p-value <0.0001(^{d}).

Key: **EGC** = Ethanol extracts of *Garcinia kola*, D. Control =Diabetic Control, Gliben. = Glibenclamide.

Table 4.3 : Effect of ethanol extracts of *Garcinia kola* on blood sugar level (BSL) of diabetic rats

Groups mg/kg	Day 0	Day 5	Day 15	Day 30
Control	86.2±0.6	85.1±1.9	91.1±0.7	87.2±0.9
D. Control	506.1±30	610.7±24	630±12	626.3±17
Gliben 5	540±3.6	149±2.3[a]	138±3.0[c]	90±1.2[d]
EGC 150	520.3±1.9	160±2.7[a]	92.4±1.2[d]	97 ±4.0[d]
EGC 500.	528±36	170±1.1[b]	29±0.5[d]	75.06±6.0[d]

Results are expressed as mean ± SEM (n= 6), with significant P-values [a,b,c,d]= p <0.05, 0.1,0.01 and 0.001

Key: EGC = Ethanol extracts of *Garcinia kola*, D. Control =Diabetic control, Gliben. = Gilbenclamide.

CHAPTER FIVE

DISCUSSION

5.1 Effect of ethanol extract of Garcinia kola on liver function indices

ALP, AST, and ALT are crucial indicators of the liver's cellular health and functionality, frequently entering the bloodstream when the liver sustains damage. Assessing the enzymatic activities of aminotransferases (AST and ALT) and phosphatases holds clinical and toxicological significance, as alterations in their activities signal potential tissue harm from toxins or diseases (Radhika et al., 2012). Conditions like cellular damage, tissue necrosis, and cardiovascular diseases can elevate ALT and AST levels in the blood (Adeyemi et al., 2015).

In the research, the study observed increased levels of diagnostic enzymes (AST, ALT, and ALP) in the serum of streptozotocin diabetic rat models, attributed to STZ toxicity affecting tissues expressing the GLUT 2 transporter, such as hepatocytes and renal tubular cells.

Additionally, the study showed a significant reduction ($p<0.05$) in serum ALT levels and a slight decrease in AST levels, implying that the ethanol extract of Garcinia kola might possess some hepato-protective properties.

5.2 Effect of ethanol extract of Garcinia kola on lipid profile indices

Plasma cholesterol levels can be controlled through various mechanisms, including cholesterol production, removal from the bloodstream, absorption from dietary sources, and elimination via bile. Additionally, LDL appears to play a role in increasing secretory phospholipase A2, contributing to atherosclerosis by infiltrating arterial wall lipids, which are then oxidized by reactive oxygen species into ox-LDL. Ox-LDL, in turn, triggers the release of phospholipids, activates endothelial cells, initiates an inflammatory cascade, and ultimately leads to the formation of foam cells and fatty streaks.

The results of this current study demonstrate a significant decrease in total cholesterol, triglyceride, and LDL concentrations in rats treated with the ethanol extract of Garcinia kola compared to the diabetic control rats. This aligns with previous research highlighting the role of HDL in managing LDL activities.

5.3 Effect of ethanol extract of Garcinia kola on blood sugar level (BSL) of Steptozotocin induced diabetic rats

The study found that the ethanol extract of Garcinia kola effectively lowered the elevated blood glucose levels in diabetic rats induced by Streptozotocin, similar to the positive effects observed with Glibenclamide, the standard drug used to treat diabetes.

As shown in Table 4.4, intravenous injection of Streptozotocin-induced a significant increase in blood glucose levels throughout the experimental period. Administration of ethanol extract of Garcinia kola at all doses results in a significant ($p \leq 0.05$, 0.1, 0.01 and 0.001) decrease of blood glucose levels near normal values by day 5. The reduction was 68.12% and 66.09% respectively at the doses of 90 and 250 mg/kg compared to the initial value. After 15 days of treatment with plant extract, the decrease in blood glucose levels was 63.12% and 84.35%. It was reported that, glibenclamide is ineffective when β-cells are destroyed (Hosseinzadeh *et al*., 2002). At the end of treatment, animals treated with Glibenclamide (5 mg/kg) have shown a decrease of 62.68% suggesting a partial destruction of pancreas β-cells while those treated with ethanol extract of Garcinia kola (90 and 250 mg/kg) showed a respectively compared to their initial value and to the diabetic control. It is known that streptozotocin acts by destructing β-cells (Szkudelski and Szkudeska, 2002); thus the plant extract could act in this condition by enhancing peripheral glucose uptake. This provides evidence that the plant extract could contain hypoglycaemic compound.

CONCLUSION

The lowering of blood glucose levels found in this research implies that Garcinia kola has hypoglycemic properties. The research, therefore suggests that Garcinia kola has the potential to be effective in controlling hyperglycemia and diabetes mellitus.

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