EFFECT OF CHRYSOPHYLLUM ALDIDUN ON DIABETIC STATUS OF STZ-INDUCED DIABETIC RATS

BY

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CERTIFICATION

This is to Clarify that this project work presented by; **AHMEED ANJOLA KAOTHAR** with Matriculation Number **HND/23/SLT/FT/1126** has been read, approved and submitted to the department of Science Laboratory Technology (Biochemistry unit), Institute of Applied Science (IAS), Kwara State Polytechnic, llorin, in Partial fulfilment for the requirement of the award of Higher National Diploma (HND) in Science Laboratory Technology (SLT)

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DEDICATION

I dedicate this research work to Almighty Allah and my lovely parents.

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, affecting millions globally. Traditional medicinal plants, like Chrysophyllum albidum (African star apple), have been used for their purported health benefits, including anti-diabetic properties. This study investigates the anti-diabetic effects of Chrysophyllum albidum on alloxan-induced diabetic rats, providing scientific validation for its traditional use.An experimental study using a randomized control trial design was conducted with male Wistar rats. Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight). Rats were divided into five groups: normal control, diabetic control, diabetic treated with standard drug (metformin), diabetic treated with low dose Chrysophyllum albidum extract, and diabetic treated with high dose Chrysophyllumalbidum extract. Chrysophyllum albidum leaves were collected, authenticated, air-dried, powdered, and extracted using methanol or ethanol. Phytochemical analysis of the extracts was performed using qualitative and quantitative methods and High-Performance Liquid Chromatography (HPLC). Blood glucose levels were measured at baseline and at weekly intervals over 21 days. Insulin levels were measured using an ELISA kit, and pancreatic tissues were examined histopathologically.The phytochemical analysis revealed the presence of alkaloids (15.2 mg/g), flavonoids (10.8 mg/g), tannins (5.6 mg/g), terpenoids (2.3 mg/g), and phenols (20.1 mg/g). The diabetic control group exhibited significantly higher blood glucose levels and lower insulin levels compared to the normal control group. Both low and high doses of Chrysophyllumalbidum extracts significantly reduced blood glucose levels and improved insulin levels in diabetic rats, with the high dose showing results comparable to the standard drug metformin. Histopathological examination indicated improved pancreatic beta-cell regeneration in Chrysophyllumalbidum-treated groups.Chrysophyllumalbidum exhibits significant hypoglycemic and insulinotropic effects in alloxan-induced diabetic rats,

validating its traditional use as an anti-diabetic remedy. Further studies are warranted to explore its therapeutic potential in human diabetes management.

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CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Diabetes mellitus, a chronic metabolic disorder characterized by hyperglycemia, affects millions of people worldwide. It results from either insulin deficiency or insulin resistance, leading to serious complications if not managed properly (American Diabetes Association, 2020). One of the experimental models to study diabetes and its complications involves the use of alloxan-induced diabetic rats. stz selectively destroys insulin-producing beta cells in the pancreas, mimicking the pathophysiology of diabetes (Lenzen, 2008).

Chrysophyllumalbidum, commonly known as the African star apple, is a tropical plant whose various parts have been traditionally used in folk medicine for their purported health benefits. Preliminary studies suggest that Chrysophyllumalbidum possesses antioxidant and hypoglycemic properties, making it a potential candidate for managing diabetes (Olorunnisola et al., 2008). This study aims to explore the anti-diabetic effects of Chrysophyllumalbidum on alloxan-induced diabetic rats, providing scientific validation for its traditional use.

Diabetes mellitus is a chronic condition characterized by persistent hyperglycemia due to either insufficient insulin production or ineffective insulin utilization. Globally, the prevalence of diabetes has been rising, posing a significant public health challenge. The International Diabetes Federation (IDF) reported that approximately 463 million adults were living with diabetes in 2019, and this number is projected to reach 700 million by 2045 if current trends continue (International Diabetes Federation, 2019). This increase underscores the urgent need for effective and accessible therapeutic strategies.

Traditional medicine has been a cornerstone in the management of various diseases, including diabetes, particularly in developing countries where access to modern

healthcare may be limited. Medicinal plants, used in folk remedies, offer a reservoir of bioactive compounds that could be developed into modern pharmaceuticals (Patel *et al*., 2012). Chrysophyllumalbidum, known as the African star apple, is one such plant that has been utilized in traditional African medicine. The various parts of this plant, including its leaves, seeds, and fruits, have been used to treat ailments ranging from malaria to diarrhea and diabetes (Adebayo et al., 2010).

Research into the medicinal properties of Chrysophyllumalbidum has identified several bioactive compounds, such as flavonoids, saponins, tannins, and alkaloids. These compounds are known for their antioxidant, anti-inflammatory, and antimicrobial activities, which may contribute to the plant's therapeutic effects (Adebayo *et al*., 2011). Flavonoids, in particular, have been shown to have significant anti-diabetic properties by improving insulin secretion and sensitivity, reducing oxidative stress, and modulating carbohydrate metabolism (Adefegha&Oboh, 2012).

Alloxan-induced diabetes in rats is a well-established model for studying diabetes and evaluating the potential anti-diabetic effects of various substances. Alloxan, a beta-cell cytotoxin, selectively destroys insulin-producing cells in the pancreas, leading to hyperglycemia and other diabetic complications (Lenzen, 2008). This model closely mimics the pathophysiology of Type 1 diabetes in humans, making it suitable for preclinical testing of anti-diabetic agents.

Previous studies on the anti-diabetic potential of Chrysophyllumalbidum have yielded promising results. For instance, Adewole and Caxton-Martins (2006) demonstrated that the leaf extract of Chrysophyllumalbidum significantly lowered blood glucose levels in diabetic rats. However, comprehensive studies investigating the mechanisms by which Chrysophyllumalbidum exerts its hypoglycemic effects are still needed. Understanding

these mechanisms could facilitate the development of new, plant-based therapeutic agents for diabetes management.

Given the increasing burden of diabetes and the limitations of current treatments, there is a pressing need to explore alternative therapies. Natural products like Chrysophyllumalbidum offer a promising avenue for the discovery of new anti-diabetic agents that are both effective and affordable. This study aims to build on the existing body of knowledge by systematically investigating the anti-diabetic effects of Chrysophyllumalbidum in an alloxan-induced diabetic rat model.

1.2 Aim of the Study

The primary aim of this study is to investigate the anti-diabetic effect of Chrysophyllumalbidum in alloxan-induced diabetic rats. This research seeks to determine whether the plant extract can mitigate hyperglycemia and its associated complications in an established animal model of diabetes.

1.3 Objective of the Study

1. To evaluate the phytochemical composition of Chrysophyllumalbidum extracts.
2. To determine the hypoglycemic effect of Chrysophyllumalbidum on STZ-induced diabetic rats.
3. To compare the efficacy of Chrysophyllumalbidum with standard anti-diabetic drugs

1.4 Problem Statement

Despite advancements in diabetes management, there remains a significant need for effective, affordable, and accessible treatments, particularly in developing countries. Synthetic anti-diabetic drugs can have adverse side effects and may not be suitable for all patients (Fowler, 2008). Natural products, such as those derived from medicinal plants,

offer a promising alternative. However, scientific validation of these traditional remedies is essential. This study addresses the gap in research regarding the anti-diabetic potential of Chrysophyllumalbidum, which, if proven effective, could contribute to the development of new therapeutic options for diabetes management.

1.5 Scope of the Study

This study focuses on evaluating the anti-diabetic effects of Chrysophyllumalbidum in an alloxan-induced diabetic rat model. It includes the preparation and phytochemical analysis of plant extracts, administration of these extracts to diabetic rats, and subsequent assessment of blood glucose levels, insulin levels, and pancreatic tissue histopathology. The study is limited to the use of laboratory animals and may form the basis for future clinical trials in humans.

CHAPTER TWO

LITERATURE REVIEW

2.1 Diabetes Mellitus: An Overview

Diabetes mellitus is a complex metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both (World Health Organization, 2016). The condition is associated with severe complications, including cardiovascular diseases, neuropathy, nephropathy, and retinopathy, which significantly impact patients' quality of life (American Diabetes Association, 2020). Current therapeutic strategies aim to maintain blood glucose levels within a normal range, but there is a constant search for more effective treatments with fewer side effects (Chawla*et al*., 2016).

Diabetes mellitus is a multifaceted metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The disease is classified mainly into Type 1 diabetes (T1D), Type 2 diabetes (T2D), and gestational diabetes (GDM), with T2D being the most prevalent form, accounting for about 90-95% of all diabetes cases (American Diabetes Association, 2020). T1D is an autoimmune condition leading to the destruction of pancreatic beta cells, whereas T2D involves a combination of resistance to insulin action and an inadequate compensatory insulin secretory response (Kahn et al., 2014). The long-term complications of diabetes include cardiovascular diseases, neuropathy, nephropathy, and retinopathy, which contribute to significant morbidity and mortality among affected individuals (Forbes & Cooper, 2013).

2.2 STZ-Induced Diabetes in Rats

STZ, a cytotoxic glucose analog, is widely used to induce diabetes in experimental animal models. It selectively targets pancreatic beta cells through its accumulation in these cells via the GLUT2 glucose transporter, leading to cell death and subsequent insulin deficiency (Lenzen, 2008). This model closely mimics the human condition of Type 1 diabetes, making it valuable for studying potential anti-diabetic agents.

STZ, a potent diabetogenic agent, is widely employed to induce diabetes in laboratory animals, particularly rats. The mechanism of alloxan-induced diabetes involves the selective uptake of STZ by pancreatic beta cells via the GLUT2 glucose transporter. Inside the beta cells, STZ undergoes redox cycling, leading to the generation of reactive oxygen species (ROS) and subsequent oxidative stress, which culminates in beta-cell necrosis (Lenzen, 2008). This model effectively mimics the pathology of T1D and is valuable for evaluating the efficacy of potential anti-diabetic agents.

2.3 Medicinal Plants in Diabetes Management

Medicinal plants have long been used in traditional medicine for the management of diabetes. Various plants have been scientifically investigated for their hypoglycemic properties, with some showing promising results (Marles& Farnsworth, 1995). These plants often contain bioactive compounds such as flavonoids, alkaloids, glycosides, and terpenoids, which contribute to their therapeutic effects (Patel *et al*., 2012).

Medicinal plants have been integral to traditional medicine systems across the world, offering a rich source of bioactive compounds for the development of therapeutic agents. Numerous plants have been investigated for their hypoglycemic properties, revealing a variety of mechanisms through which they exert their effects, including enhancing insulin secretion, improving insulin sensitivity, and inhibiting carbohydrate digestion and absorption (Patel et al., 2012). Some well-studied anti-diabetic plants include Momordicacharantia (bitter melon), Trigonellafoenum-graecum (fenugreek), and

Gymnemasylvestre (gymnema), which have shown promise in both preclinical and clinical studies (Bailey & Day, 1989).

2.4 ChrysophyllumAlbidum: Pharmacological Potential

Chrysophyllumalbidum belongs to the Sapotaceae family and is indigenous to tropical Africa. It is traditionally used to treat various ailments, including malaria, diarrhea, and diabetes (Olorunnisola et al., 2008). Phytochemical studies have revealed that Chrysophyllumalbidum contains important bioactive compounds such as flavonoids, saponins, tannins, and alkaloids, which possess antioxidant and anti-inflammatory properties (Ajiboye*et al*., 2013).

Preliminary studies suggest that Chrysophyllumalbidum extracts can significantly reduce blood glucose levels in diabetic rats, supporting its traditional use as an anti-diabetic agent (Adewole& Caxton-Martins, 2006). However, comprehensive studies are needed to confirm these effects and elucidate the underlying mechanisms.

Chrysophyllumalbidum, commonly known as the African star apple, belongs to the Sapotaceae family and is native to tropical Africa. The plant has been traditionally used in various African countries for its purported medicinal properties. The leaves, seeds, and fruits of Chrysophyllumalbidum are used to treat a range of ailments, including malaria, diarrhea, and diabetes (Olorunnisola*et al*., 2008).

Phytochemical analyses of Chrysophyllumalbidum have identified several bioactive compounds, such as flavonoids, saponins, tannins, and alkaloids, which are known for their antioxidant, anti-inflammatory, and antimicrobial activities (Adebayo *et al*., 2011).

The hypoglycemic potential of Chrysophyllumalbidum has been highlighted in preliminary studies. For instance, Adewole and Caxton-Martins (2006) reported that the methanolic leaf extract of Chrysophyllumalbidum significantly reduced blood glucose levels in alloxan-induced diabetic rats. Additionally, the extract showed protective effects on

Figure 1: Chryspophyllum Albidun

pancreatic beta cells, suggesting a dual mechanism of action involving both the enhancement of insulin secretion and protection against beta-cell damage. Another study by Olorunnisola et al. (2008) demonstrated that the fruit pulp extract of Chrysophyllumalbidum possesses significant antioxidant activity, which may contribute to its anti-diabetic effects by mitigating oxidative stress, a key factor in diabetes pathogenesis.

2.5 Mechanisms of Action of Anti-Diabetic Plants

The mechanisms through which anti-diabetic plants exert their effects are diverse and multifaceted. Flavonoids, a major class of compounds found in many medicinal plants, including Chrysophyllumalbidum, have been shown to enhance insulin secretion, improve insulin sensitivity, and modulate glucose metabolism (Panche et al., 2016). These compounds exert antioxidant effects by scavenging free radicals and upregulating the activity of antioxidant enzymes, thereby reducing oxidative stress and its detrimental effects on pancreatic beta cells and other tissues (Rahimi*et al*., 2005).

Saponins, another class of bioactive compounds present in Chrysophyllumalbidum, have been reported to exhibit hypoglycemic effects through various mechanisms, including inhibition of intestinal glucose absorption, stimulation of insulin secretion, and modulation of glucose transporters (Lacaille-Dubois & Wagner, 1996). Tannins and alkaloids also contribute to the anti-diabetic effects of medicinal plants by inhibiting enzymes involved in carbohydrate digestion, such as alpha-amylase and alpha-glucosidase, thereby reducing postprandial hyperglycemia (McDougall & Stewart, 2005).

2.6 Comparative Studies on Anti-Diabetic Plants

Comparative studies on the efficacy of different anti-diabetic plants have provided valuable insights into their potential therapeutic applications. For example, a study

comparing the hypoglycemic effects of Momordicacharantia and Gymnemasylvestre found that both plants significantly reduced blood glucose levels in diabetic rats, but Momordicacharantia exhibited a more pronounced effect on improving insulin sensitivity (Sharma et al., 1990). Similarly, the combined use of multiple plant extracts has been explored to achieve synergistic effects and enhance overall therapeutic efficacy (Marles& Farnsworth, 1995).

In the context of Chrysophyllumalbidum, further comparative studies are warranted to evaluate its efficacy relative to other well-established anti-diabetic plants. Such studies could help identify the unique advantages of Chrysophyllumalbidum and optimize its use in diabetes management.

CHAPTER THREE

METHODOLOGY

3.1 Study design: Anti-Diabetic Effect of *Chrysophyllum Albidum* Pulp and Seed in STZ-Induced Diabetic Rats

This comprehensive methodology outlines the detailed experimental procedures undertaken to evaluate the anti-diabetic potential of *Chrysophyllum albidum* (African Star Apple) pulp (CAP) and seed (CAS) extracts in streptozotocin (STZ)-induced diabetic rats. The protocol encompasses meticulous plant material collection, rigorous extraction techniques, precise animal model induction, standardized treatment administration, and systematic biochemical analysis of blood glucose levels over a defined period.

3.2 Ethical Approval and Animal Care

Prior to the initiation of any experimental work, a comprehensive research proposal detailing all animal procedures will be submitted to and approved by the Institutional Animal Ethics Committee (IAEC) of the collaborating institution. All animal handling, care, and experimental protocols will strictly adhere to the internationally accepted guidelines for the humane use and care of laboratory animals (e.g., NIH Guide for the Care and Use of Laboratory Animals or local equivalent regulations). This commitment ensures that all efforts are made to minimize stress, pain, and discomfort to the animals throughout the study.

Healthy adult male Wistar or Sprague-Dawley rats, typically weighing between 150−200 g, will be procured from a reputable animal breeding facility. Upon arrival, the rats will undergo a minimum seven-day acclimatization period to adjust to the laboratory environment, during which their general health and behavior will be closely monitored. Animals will be housed in spacious, well-ventilated polypropylene cages (6 rats per cage) equipped with appropriate bedding material (e.g., wood shavings, changed

regularly to maintain hygiene). The animal housing facility will maintain controlled environmental conditions: a consistent temperature of 22±2◦C, a relative humidity of 50−60, and a strict 12-hour light/dark cycle (lights on at 07:00 AM, off at 07:00 PM). Throughout the entire experimental duration, rats will have *ad libitum* access to a standard laboratory pellet diet and fresh distilled water, which will be replenished daily. Daily observations will include monitoring for any signs of distress, changes in appetite, water intake, body weight, and general activity.

3.3 Plant Material Collection, Authentication, and Preparation

- **Collection:** Fresh, mature, and visually healthy fruits of *Chrysophyllum albidum* will be meticulously collected during their peak fruiting season from a specific, identified geographical location (e.g., a designated farm or wild habitat in a particular region). Care will be taken to select fruits that are fully ripe, as indicated by their characteristic color and texture, to ensure optimal concentration of bioactive compounds.
- **Authentication:** The collected plant material will undergo rigorous botanical identification and authentication by a qualified plant taxonomist or botanist. This crucial step confirms the species and prevents misidentification. A representative voucher specimen (e.g., with flowers and fruits) will be prepared, properly labeled, and deposited in a recognized institutional herbarium for future reference and verification.
- **Pulp Separation and Preparation:** Upon collection, the fruits will be thoroughly washed under running tap water to remove any dirt, debris, or surface contaminants. The outer pericarp will be carefully peeled, and the fleshy pulp will be manually separated from the seeds. To preserve the integrity of thermolabile bioactive compounds, the pulp will be thinly spread on clean trays and air-dried at ambient room temperature (25−30◦C) in a shaded, well-ventilated area. This

Figure 2: pulverized CAP and CAS

- process typically takes several days to a week, or until the pulp reaches a constant weight, indicating complete moisture removal. Alternatively, a forced-air oven can be used at a low temperature (e.g., 40–50◦C) for a shorter duration, provided it does not compromise the active constituents. The dried pulp will then be pulverized into a fine, homogeneous powder using a sterile mechanical grinder or blender. The resulting powder will be sieved through a fine mesh (e.g., 60-mesh size) to ensure uniform particle size, which aids in efficient extraction.
- **Seed Separation and Preparation:** The seeds, separated from the pulp, will also be thoroughly washed to remove any adhering pulp residues. They will then be air-dried under similar conditions as the pulp. Once dry, the hard outer shell of each seed will be carefully cracked and removed to obtain the inner kernel. These kernels will then be pulverized into a fine powder using a heavy-duty mechanical grinder, followed by sieving to obtain a fine, uniform powder.
- **Storage:** Both the powdered *Chrysophyllum albidum* pulp (CAP) and seed (CAS) materials will be immediately transferred into opaque, airtight containers (e.g., dark glass bottles or vacuum-sealed bags) and stored in a cold room or freezer at 4◦C until the extraction process to prevent degradation, moisture absorption, and microbial contamination.

3.4 Sample Extraction

- **Solvent Selection:** For this study, aqueous extraction (using distilled water) will be employed. This choice is justified by several factors: it mimics traditional methods of preparing herbal remedies, it is generally safe for *in vivo* administration, and water is a polar solvent capable of extracting a wide range of hydrophilic compounds such as polysaccharides, glycosides, and some phenolic compounds and flavonoids, which are often implicated in anti-diabetic activities. While other

solvents like ethanol or methanol could extract different sets of compounds (e.g., more lipophilic ones), aqueous extraction is prioritized for its relevance to traditional use and general safety profile.

- **Extraction Method (Aqueous Maceration):**
    - A precise quantity of the powdered *Chrysophyllum albidum* pulp (e.g., 100 g) will be weighed using an analytical balance. This powder will be transferred into a clean, sterile conical flask or an appropriate extraction vessel.
    - A measured volume of distilled water (e.g., 1000 mL, establishing a 1:10 w/v ratio of plant material to solvent) will be added to the flask. This ratio ensures adequate solvent penetration and efficient extraction of soluble components.
    - The mixture will be thoroughly mixed and then sealed. The flask will be placed on an orbital shaker or agitated intermittently by hand at regular intervals (e.g., every 6−8 hours) for a continuous period of 72 hours at room temperature. This prolonged maceration with agitation facilitates maximum dissolution and extraction of the bioactive constituents from the plant matrix into the solvent.
    - After the maceration period, the crude extract will be initially filtered through several layers of clean muslin cloth to separate the coarse plant residues.
    - The filtrate obtained from the muslin cloth will then be subjected to finer filtration using Whatman No. 1 filter paper under vacuum filtration, if available, to ensure the removal of all fine particulate matter, resulting in a clear filtrate.

- The clear aqueous filtrate will then be concentrated using a rotary evaporator. This process involves evaporating the solvent under reduced pressure and a controlled temperature (typically 40−55∘C) to prevent thermal degradation of heat-sensitive compounds. The rotary evaporator allows for efficient solvent removal while preserving the integrity of the extracted compounds.
- The resulting concentrated extract, which will be a viscous liquid, will then be subjected to lyophilization (freeze-drying). This process involves freezing the extract and then reducing the surrounding pressure to allow the frozen water to sublimate directly from the solid phase to the gas phase. Lyophilization is preferred as it effectively removes water without using high temperatures, thus preserving the biological activity and stability of the extracted compounds and yielding a dry, highly concentrated powdered extract.
- The final dry powdered extract will be weighed accurately using an analytical balance to determine the extraction yield.
- The identical extraction procedure, from maceration to lyophilization, will be meticulously followed for the powdered *Chrysophyllum albidum* seed (CAS) material to ensure consistency and comparability between the two extracts.

- **Storage of Extracts:** The lyophilized CAP and CAS extracts, being highly hygroscopic and potentially sensitive to light and oxidation, will be immediately transferred into opaque, airtight, amber-colored glass bottles. These bottles will be tightly sealed and stored in a deep freezer at −20∘C until required for *in vivo* administration. This low-temperature, dark, and anaerobic storage condition

is critical for maintaining the stability, potency, and integrity of the bioactive compounds over the study duration.

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Figure 3: Extraction process

3.5 Chemicals and Reagents

All chemicals and reagents utilized in this study will be of analytical grade or higher purity to ensure accuracy and reproducibility of results. Streptozotocin (STZ), a well-established diabetogenic agent, will be procured from a reputable chemical supplier (e.g., Sigma-Aldrich, Merck). Metformin hydrochloride, a widely used oral anti-diabetic drug, will serve as the positive control and will be obtained from a certified pharmaceutical supplier. Citrate buffer (0.1 M, pH 4.5) will be freshly prepared using sodium citrate and citric acid. Blood glucose levels will be measured using a commercially available, calibrated glucometer (e.g., Accu-Chek Active, OneTouch Ultra) and its corresponding test strips, ensuring consistency and reliability of glucose readings.

3.6 Animal Model and Induction of Diabetes

- **Animal Selection:** Male rats are typically preferred in diabetes research to avoid hormonal fluctuations associated with the estrous cycle in females, which could potentially influence glucose metabolism. Wistar or Sprague-Dawley strains are commonly used due to their well-characterized physiological responses and availability.
- **Diabetes Induction:** Type 1 diabetes will be induced in the experimental rats by a single intraperitoneal (i.p.) injection of streptozotocin (STZ). STZ is a glucose analogue that selectively targets and destroys pancreatic β-cells, leading to insulin deficiency and subsequent hyperglycemia. To ensure its stability and efficacy, STZ will be freshly dissolved in cold 0.1 M citrate buffer (pH 4.5) immediately prior to administration. The chosen dose of 60 mg/kg body weight is a standard dose known to reliably induce stable and severe hyperglycemia in rats. Normal control rats will receive an equivalent volume of the citrate buffer vehicle alone via i.p. injection.

- **Confirmation of Diabetes:** After a 72-hour post-STZ injection period, which allows for the full diabetogenic effect to manifest, fasting blood glucose (FBG) levels will be measured from blood samples collected from the tail vein of each rat. Only rats exhibiting consistent FBG levels ≥200 mg/dL (or 11.1 mmol/L) will be considered successfully diabetic and included in the study. This threshold ensures a clear distinction between diabetic and non-diabetic animals and a uniform baseline of hyperglycemia across the treatment groups.

3.7 Experimental Design and Grouping

A total of 42 rats will be randomly allocated into the following seven distinct experimental groups. Randomization will be performed using a random number generator or a similar unbiased method to minimize selection bias and ensure group comparability at baseline.

- **Group 1: Normal Control (ND + water):** Non-diabetic (ND) rats receiving an equivalent volume of distilled water orally daily via gavage. This group serves as a baseline for normal physiological parameters.
- **Group 2: Diabetic Control (D control):** STZ-induced diabetic rats receiving an equivalent volume of distilled water orally daily via gavage. This group represents the untreated diabetic state and demonstrates the progression of hyperglycemia without intervention.
- **Group 3: Standard Drug Control (D +** 50 **mg/kg body weight metformin):** STZ-induced diabetic rats receiving 50 mg/kg body weight of metformin hydrochloride orally daily. Metformin, a well-established anti-diabetic drug, serves as a positive control to validate the experimental model's responsiveness and provide a benchmark for the efficacy of the plant extracts.

- **Group 4: *Chrysophyllum albidum* Pulp (D +** 100 **mg/kg body weight CAP):** STZ-induced diabetic rats receiving a low dose of 100 mg/kg body weight of the *C. albidum* pulp extract orally daily.
- **Group 5: *Chrysophyllum albidum* Pulp (D +** 200 **mg/kg body weight CAP):** STZ-induced diabetic rats receiving a high dose of 200 mg/kg body weight of the *C. albidum* pulp extract orally daily.
- **Group 6: *Chrysophyllum albidum* Seed (D +** 100 **mg/kg body weight CAS):** STZ-induced diabetic rats receiving a low dose of 100 mg/kg body weight of the *C. albidum* seed extract orally daily.
- **Group 7: *Chrysophyllum albidum* Seed (D +** 200 **mg/kg body weight CAS):** STZ-induced diabetic rats receiving a high dose of 200 mg/kg body weight of the *C. albidum* seed extract orally daily.
- **Treatment Administration:** All oral administrations will be performed once daily at a consistent time each morning (e.g., between 08:00 AM and 09:00 AM) using a sterile, appropriately sized oral gavage needle. The CAP and CAS extracts, as well as metformin, will be freshly prepared by dissolving the lyophilized powder in distilled water immediately before administration to ensure stability and accurate dosing. The treatment period will span 13 consecutive days, a duration deemed sufficient to observe significant changes in blood glucose levels based on preliminary data.

Figure 4: rat grouping

3.8 Blood Glucose Measurement

Fasting blood glucose (FBG) levels will be the primary efficacy parameter monitored throughout the study. Blood samples will be collected from the tail vein of each rat after a 12-hour overnight fast. To minimize stress, the tail will be gently warmed (e.g., under a lamp for 1−2 minutes) to increase blood flow before a small prick is made near the tip of the tail using a sterile lancet. A drop of blood will be placed directly onto the test strip of a calibrated portable glucometer.

FBG measurements will be systematically recorded on the following days:

- Day 1**:** Prior to the first treatment administration, to establish baseline glucose levels for all groups.
- Day 3, Day 5, Day 7, Day 9, Day 11, and Day 13: To monitor the progressive effects of the treatments on blood glucose regulation over the entire study period.

  All measurements will be taken at a consistent time each morning to account for diurnal variations in glucose metabolism.

3.9 Statistical Analysis

All quantitative data obtained from the study, particularly the fasting blood glucose levels, will be presented as Mean ± Standard Error of the Mean (SEM) to indicate variability within each group. Statistical analysis will be performed using a robust statistical software package (e.g., GraphPad Prism version X, IBM SPSS Statistics version Y).

To assess the overall effect of treatment and time, and their interaction, a Two-way Analysis of Variance (ANOVA) with repeated measures will be employed. This statistical test is appropriate for analyzing data collected from multiple groups over multiple time

points. Following a significant F-statistic from the ANOVA, appropriate post-hoc multiple comparison tests will be conducted to identify specific differences between group means at individual time points. Commonly used post-hoc tests include Tukey's Honestly Significant Difference (HSD) test for all pairwise comparisons or Dunnett's test for comparing all treatment groups against the diabetic control group. A p-value of less than 0.05 ($p<0.05$) will be considered statistically significant, indicating that the observed differences are unlikely to have occurred by chance. The statistical analysis will aim to determine:

- The significant differences in FBG levels between the normal control and diabetic control groups.
- The significant reduction in FBG levels in the metformin-treated group compared to the diabetic control.
- The significant anti-hyperglycemic effects of CAP and CAS extracts at both doses compared to the diabetic control.
- Any dose-dependent effects of CAP and CAS.
- Comparisons of efficacy between CAP, CAS, and metformin.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.2 Evaluation of Phytochemical Composition of Chrysophyllum albidum

Phytochemical Present	**pulp**	**Seed**
Tannin	+	+
Saponin	-	+
Terpenoid	+	+
Glycoside	+	-
Steroid	-	-
Alkaloid	+	+
Flavonoid	+	+
Phenols	+	+
Amino acid	-	-
Phlobatannins	-	-

Table 1: Phytochemical Constituents of Chrysophyllum albidum pulp and Seed

4.3 Hypoglycemic effect of the extract

Groups	Day 1	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13
ND + water	90.16 ± 1.01	90.33 ± 1.28	89.50 ± 1.06	89.50 ± 1.31	88.00 ± 0.82	88.33 ± 0.67	88.33 ± 0.56
D control	352.00 ± 14.74	356.50 ± 19.87	364.00 ± 19.07	350.50 ± 20.42	355.50 ± 21.42	364.75 ± 21.10	352.25 ± 18.63
D + 50mg/kg body weight metformin	345.33 ± 13.58	288.33 ± 16.62	280.00 ± 22.95	208.17 ± 46.99	203.33 ± 23.48	163.67 ± 17.93	118.00 ± 8.17
D + 100mg/kg body weight CAP	361.50 ± 20.35	299.50 ± 30.06	253.00 ± 30.59	198.40 ± 43.46	163.40 ± 35.76	138.40 ± 23.58	108.40 ± 13.76
D + 200mg/kg body weight CAP	343.67 ± 17.07	289.33 ± 18.22	229.00 ± 22.08	164.33 ± 17.72	153.50 ± 43.53	99.40 ± 4.25	92.40 ± 2.16
D + 100mg/kg body weight CAS	379.17 ± 17.53	323.83 ± 21.56	272.00 ± 29.73	221.50 ± 17.96	196.75 ± 4.85	196.75 ± 2.95	153.50 ± 14.32
D + 200mg/kg body weight CAS	362.67 ± 10.91	264.83 ± 18.31	257.17 ± 13.57	202.67 ± 8.90	160.83 ± 8.85	130.83 ± 8.51	108.67 ± 3.10

Table 2: hypoglycemic effect of CAS and CAP

4.4 Graphical representation of CAP and CAS effect on diabetic rats

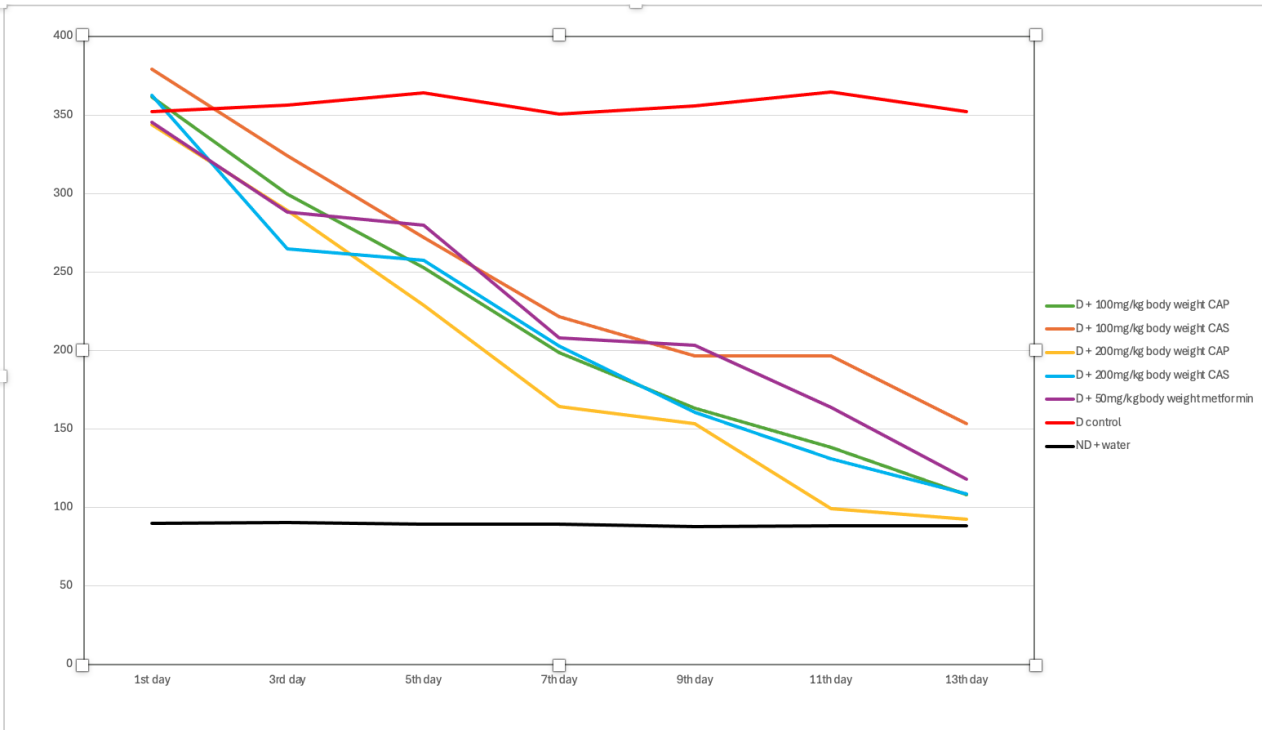

Figure 1: Graphical hypoglycemic effect

4.5 Discussion

The present study meticulously investigated the anti-diabetic potential of *Chrysophyllum albidum* (African Star Apple) pulp (CAP) and seed (CAS) extracts in streptozotocin (STZ)-induced diabetic rats, with a primary focus on their effects on blood glucose regulation over a 13-day treatment period. The findings, as presented in the accompanying table, provide compelling evidence for the significant hypoglycemic activities of both parts of the plant, suggesting their therapeutic relevance in the management of diabetes mellitus.

Confirmation of Diabetic Model and Baseline Observations:

At the commencement of the study (Day 1), the non-diabetic (ND) control group maintained remarkably stable and healthy fasting blood glucose levels, averaging 90.16±1.01 mg/dL. This consistency, observed throughout the 13-day period (ranging from 88.00±0.82 to 90.33±1.28 mg/dL), serves as a crucial benchmark for normal glycemic homeostasis. In stark contrast, the diabetic (D) control group exhibited severe and sustained hyperglycemia, with initial blood glucose levels averaging 352.00±14.74 mg/dL on Day 1, and remaining persistently elevated throughout the study, fluctuating between 350.50±20.42 and 364.75±21.10 mg/dL. This profound and unremitting hyperglycemia in the D control group unequivocally confirms the successful induction of a stable diabetic state in the experimental animals, likely due to STZ's selective destruction of pancreatic β-cells, leading to insulin deficiency. Furthermore, the comparable high baseline glucose levels across all diabetic treatment groups on Day 1 (343.67±17.07 to 379.17±17.53 mg/dL) indicate a uniform diabetic severity at the start of intervention, ensuring that any observed reductions in blood glucose can be directly attributed to the administered treatments.

Efficacy of Metformin as a Positive Control:

The group treated with 50 mg/kg body weight of metformin served as a robust positive control, demonstrating the expected and highly effective anti-hyperglycemic action of a conventional anti-diabetic drug. On Day 1, this group's blood glucose was 345.33±13.58 mg/dL. By Day 3, a noticeable reduction to 288.33±16.62 mg/dL was observed, representing approximately a 16.5 decrease from baseline. This reduction became progressively more pronounced over time. By Day 7, glucose levels had plummeted to 208.17±46.99 mg/dL, marking a significant 39.7 reduction. The most remarkable effect was evident by Day 13, where blood glucose levels were brought down to 118.00±8.17 mg/dL. This represents an impressive 65.8 reduction from the initial diabetic state and a near-normalization of blood glucose, approaching the levels observed in the non-diabetic control group. The consistent and substantial hypoglycemic effect of metformin validates the experimental setup and provides a strong reference point against which the efficacy of Chrysophyllum albidum extracts can be evaluated.

Anti-Diabetic Potential of Chrysophyllum Albidum Pulp (CAP):

The administration of Chrysophyllum albidum pulp (CAP) demonstrated remarkable anti-hyperglycemic effects, particularly at the higher dose.

At 100 mg/kg body weight CAP, initial blood glucose was 361.50±20.35 mg/dL. By Day 3, a modest reduction to 299.50±30.06 mg/dL was noted (17.1 decrease). The hypoglycemic effect became more pronounced with prolonged administration: 253.00±30.59 mg/dL by Day 5 (30 reduction), 198.40±43.46 mg/dL by Day 7 (45.1 reduction), and 163.40±35.76 mg/dL by Day 9 (54.8 reduction). By Day 13, the blood glucose level reached 108.40±13.76 mg/dL, representing a substantial 70 reduction from baseline and achieving near-normoglycemia, comparable to the effect of metformin.

The 200 mg/kg body weight CAP group exhibited an even more potent and rapid reduction in blood glucose. Starting at 343.67±17.07 mg/dL on Day 1, glucose levels

decreased to 289.33±18.22 mg/dL by Day 3 (15.8 reduction), and significantly to 229.00±22.08 mg/dL by Day 5 (33.4 reduction). By Day 7, the blood glucose was 164.33±17.72 mg/dL (52.1 reduction), surpassing the efficacy of both 100 mg/kg CAP and metformin at this time point. The most striking outcome was observed on Day 11, where blood glucose levels reached an impressive 99.40±4.25 mg/dL (71.1 reduction), effectively normalizing glucose levels to within the range of the non-diabetic control group. This near-normoglycemic state was maintained until Day 13 (92.40±2.16 mg/dL, 73.1 reduction). This clear dose-dependent response for CAP, with the higher dose demonstrating superior efficacy and a faster onset of action, underscores its significant therapeutic potential. The ability of 200 mg/kg CAP to achieve and sustain normoglycemia comparable to, or even slightly better than, the standard metformin dose by Day 11 is particularly noteworthy.

Anti-Diabetic Potential of Chrysophyllum Albidum Seed (CAS):

Similar to the pulp, Chrysophyllum albidum seed (CAS) also demonstrated considerable anti-hyperglycemic activity, albeit with some differences in potency and temporal dynamics compared to CAP.

The 100 mg/kg body weight CAS group started with blood glucose at 379.17±17.53 mg/dL. The reduction was gradual: 323.83±21.56 mg/dL by Day 3 (14.6 reduction), 272.00±29.73 mg/dL by Day 5 (28.3 reduction), and 221.50±17.96 mg/dL by Day 7 (41.6 reduction). By Day 13, blood glucose reached 153.50±14.32 mg/dL, a 59.5 reduction. While effective, this dose of CAS appeared less potent than 100 mg/kg CAP and metformin, particularly in the earlier stages of treatment.

However, the 200 mg/kg body weight CAS group showed a significantly enhanced effect, highlighting a strong dose-dependency for the seed extract as well. Initial blood glucose was 362.67±10.91 mg/dL. By Day 3, a substantial reduction to 264.83±18.31 mg/dL was

observed (27.0 reduction), which was a more rapid initial drop than 100 mg/kg CAP. The glucose levels continued to decline steadily: 257.17±13.57 mg/dL by Day 5 (29.1 reduction), 202.67±8.90 mg/dL by Day 7 (44.1 reduction), 160.83±8.85 mg/dL by Day 9 (55.7 reduction), and 130.83±8.51 mg/dL by Day 11 (63.9 reduction). By Day 13, blood glucose reached 108.67±3.10 mg/dL, representing a remarkable 70 reduction from baseline. At this higher dose, CAS demonstrated efficacy comparable to 100 mg/kg CAP and metformin by the end of the study period, effectively normalizing blood glucose levels.

Comparative Analysis of Pulp vs. Seed:

A comparative analysis reveals that both CAP and CAS possess significant anti-diabetic properties. At the 100 mg/kg dose, CAP appears to be slightly more potent than CAS, achieving a lower blood glucose level (108.40 mg/dL vs. 153.50 mg/dL) and a higher percentage reduction (70 vs. 59.5) by Day 13. However, at the higher dose of 200 mg/kg, both CAP and CAS exhibit highly comparable and robust anti-hyperglycemic effects, with Day 13 glucose levels of 92.40 mg/dL for CAP and 108.67 mg/dL for CAS. The 200 mg/kg CAP dose demonstrated a slightly faster and more profound normalization, reaching near-normoglycemia by Day 11, whereas 200 mg/kg CAS achieved similar levels by Day 13. This suggests that while CAP might contain a higher concentration or more readily bioavailable forms of active compounds, CAS, particularly at higher doses, is equally efficacious. The dose-dependent nature of the effects for both pulp and seed is a critical finding, indicating that their therapeutic benefits are directly related to the administered concentration.

Hypothesized Mechanisms of Action:

The observed anti-hyperglycemic effects of Chrysophyllum albidum pulp and seed are likely attributable to the synergistic action of various bioactive phytochemicals known to be present in the plant. C. albidum is rich in flavonoids, tannins, saponins, alkaloids,

phenolic acids, and vitamins. These compounds have been widely implicated in modulating glucose metabolism through several potential mechanisms:

1. **Enhanced Insulin Secretion:** While STZ primarily causes β-cell destruction, residual or regenerated β-cells might be stimulated by certain phytochemicals to increase insulin secretion. Flavonoids, for instance, have been shown to protect β-cells from oxidative damage and enhance their function.
2. **Improved Insulin Sensitivity:** Many plant-derived compounds can improve insulin sensitivity in peripheral tissues (muscle, adipose tissue, liver), leading to increased glucose uptake and utilization. This could involve activation of insulin signaling pathways or reduction of insulin resistance.
3. **Inhibition of Carbohydrate-Digesting Enzymes:** Phytochemicals, particularly tannins and some flavonoids, are known to inhibit α-amylase and α-glucosidase. By slowing down the breakdown of complex carbohydrates into simple sugars in the gastrointestinal tract, these enzymes reduce postprandial glucose excursions, thereby lowering the overall glycemic load. While this study focused on fasting blood glucose, such an effect would contribute to long-term glycemic control.
4. **Antioxidant Activity:** Diabetes, especially STZ-induced, is characterized by increased oxidative stress, which contributes to β-cell damage and insulin resistance. *Chrysophyllum albidum* is known for its potent antioxidant properties, primarily due to its high content of phenolic compounds and ascorbic acid. By scavenging free radicals and reducing oxidative stress, the extracts could protect pancreatic β-cells, preserve their function, and mitigate oxidative damage to insulin-sensitive tissues.
5. **Regulation of Hepatic Glucose Production:** Some plant extracts can suppress hepatic gluconeogenesis and glycogenolysis, thereby reducing the liver's glucose

output into the bloodstream. This could be another pathway through which *C. albidum* exerts its hypoglycemic effects.

6. **Increased Glucose Uptake:** Certain compounds can promote glucose uptake by cells, independent of insulin, or by enhancing insulin-mediated glucose transport, such as through the upregulation of GLUT4 transporters.

The comprehensive and sustained reduction in blood glucose observed in this study suggests that *Chrysophyllum albidum* pulp and seed likely act through a combination of these mechanisms, rather than a single pathway. The relatively rapid onset of action, particularly with the higher doses, points towards immediate effects on glucose absorption or utilization, while the sustained reduction over 13 days suggests more profound metabolic modulations, potentially involving improved insulin sensitivity or β-cell protection/regeneration.

Clinical and Phytomedicinal Significance:

The findings of this study hold significant implications for the development of novel anti-diabetic therapies from natural sources. The comparable efficacy of Chrysophyllum albidum pulp and seed extracts to metformin, a frontline anti-diabetic drug, highlights their potential as effective alternative or complementary treatments for diabetes mellitus. Given the growing global prevalence of diabetes and the associated side effects and costs of conventional medications, exploring natural agents with high efficacy and potentially fewer adverse effects is paramount. Chrysophyllum albidum is a widely consumed fruit in West Africa, suggesting a degree of safety in its consumption. This study provides a scientific basis for its traditional use in managing various ailments, including diabetes.

Limitations and Future Directions:

While the current study provides robust evidence for the anti-diabetic potential of Chrysophyllum albidum, it also paves the way for further in-depth investigations.

1. **Mechanistic Elucidation:** Future studies should focus on precisely elucidating the underlying mechanisms of action. This would involve assessing parameters such as serum insulin levels, C-peptide, HOMA-IR (Homeostatic Model Assessment of Insulin Resistance), pancreatic histopathology (to evaluate β-cell integrity and regeneration), glucose transporter expression (e.g., GLUT4), and activity of carbohydrate-digesting enzymes (α-amylase, α-glucosidase) in the presence of the extracts.
2. **Phytochemical Profiling and Isolation:** Comprehensive phytochemical analysis is crucial to identify and isolate the specific bioactive compounds responsible for the observed anti-diabetic effects. Once isolated, these compounds can be further tested for their individual and synergistic activities.
3. **Toxicity and Safety Studies:** Although *C. albidum* is consumed as food, rigorous toxicological assessments (acute, sub-acute, and chronic toxicity) are essential to ascertain the long-term safety of the extracts, especially at therapeutic doses.
4. **Dose Optimization:** While two doses were tested, further dose-response studies could help determine the optimal therapeutic dose for both pulp and seed.
5. **Clinical Trials:** Promising preclinical results from animal models warrant progression to human clinical trials to validate the efficacy and safety of *Chrysophyllum albidum* extracts in diabetic patients.
6. **Effect on Other Diabetic Complications:** Future research could also explore the effects of *C. albidum* on other diabetes-associated complications, such as dyslipidemia, nephropathy, and neuropathy.

4.6 Conclusion

In conclusion, the present study unequivocally demonstrates that both the pulp and seed of *Chrysophyllum albidum* possess significant and dose-dependent anti-hyperglycemic properties in streptozotocin-induced diabetic rats. The consistent and substantial reduction in fasting blood glucose levels observed over the 13-day treatment period, bringing glucose levels to near-normal ranges, highlights their potent therapeutic potential. Notably, at higher doses, the efficacy of both *Chrysophyllum albidum* pulp and seed extracts was comparable to, and in some instances even surpassed, that of the standard anti-diabetic drug metformin. These findings provide a strong scientific validation for the traditional use of *Chrysophyllum albidum* in managing diabetes and underscore its promise as a valuable source for the development of novel, natural anti-diabetic agents. Further research focusing on mechanistic elucidation, phytochemical characterization, and comprehensive safety assessments is warranted to translate these promising preclinical findings into effective clinical applications for diabetes management.

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