**LIVER FUNCTION OF DIABETIC RATS TREATED WITH ETHANOL EXTRACT OF *Chrysophyllum albidum*(Agbalumo) STEM BARK**

**BY**

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## CERTIFICATION

We the undersigned hereby certify that this project was carried out by **ABDULMUMUNI ABDULWAHAB** with matriculation number **LSC2209199** of the department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City and this work is considered adequate for consideration in partial fulfilment of the requirements for the award of Bachelor of Science (B.Sc) in Biochemistry.

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# DEDICATION

This work is dedicated to Almighty ALLAH for His unfailing love guidance, wisdom and understanding. A special dedication to my number one supporter and father; MR ABDULMUMUNI SANNI for their immense support, love, prayers and care and also to my lovely siblings.

## ACKNOWLEDGEMENT

My greatest gratitude to Almighty Allah the most merciful, most beneficial who all praise and love is from and unto him alone. I will forever give him all my praise and affection. A special gratitude to my project supervisor, DR. OSAHON D. ABU who guidance helped me to coordinate my project. A Special appreciation to my parents, and sibling for their words of encouragement and supports. I am indebted to my amazing friends and colleagues who contributed to the success of this work.

**TABLE OF CONTENT**

## FIGURES

## ABSTRACT

This study evaluated liver function of diabetic rat treated with ethanol extract of *Chrysophyllum albidum* stem back. Diabetes was induced intraperitoneally in the rat with streptozotocin [STZ, 40mg/kg body weight (bwt)], and rats with fasting blood glucose levels exceeding 200 mg/dL were considered diabetic. Animals were treated with 200 mg/kg bwt ethanol extract of *C. albidum* stem bark or 50 mg/kg bwt metformin for 14 days, with a diabetic control group receiving no treatment. Fasting blood glucose levels were monitored using an Accu-check glucometer before and during treatment. Blood sample collected after euthenization were used for biochemical analysis. Results showed a significant reduction in blood glucose level in metformin group compared to Ethanol extract of *C.albidum* ($p < 0.05$). Activities of Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) in the plasma shown no significant increase when compared to control ($p>0.05$). Similarly, Albumin and Bilirubin concentration show no significant increase ($p>0.05$). These findings suggest that ethanol extract of C. albidum stem bark particularly at 200 mg/kg bwt, exerts significant ethnomedicinal effect on diabetic rat liver.

# CHAPTER ONE
# INTRODUCTION AND LITERATURE REVIEW

## 1.0 Background of Studies

Diabetes mellitus is a chronic metabolic disorder characterised by hyperglycaemia, which often leads to oxidative stress and hepatic dysfunction due to elevated reactive oxygen species (ROS) and lipid peroxidation (Afolabi *et al*., 2021). In streptozotocin (STZ) induced diabetic rat models, liver function is typically impaired, as evidenced by increased activities of hepatic enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), alongside reduced antioxidant enzyme levels like superoxide dismutase (SOD) and catalase (CAT).

Traditional medicinal plants, like *Chrysophyllum albidum* (African star apple), have been explored for their potential hepatoprotective and antidiabetic properties. Specifically, the ethanol extract of C. albidum stem bark has shown promising effects in ameliorating these diabetes-induced liver alterations. Studies have demonstrated that oral administration of ethanolic stem bark extract of C. albidum (EECA) at doses ranging to 200 mg/kg body weight significantly restores liver function parameters in Streptozotocin -induced diabetic Wistar rats.

For instance, in Streptozotocin-induced diabetic rats, *Chrysophyllum Albidum* prevented hyperglycemia and modulated serum enzyme markers for liver function, including reductions in elevated ALT, AST, and ALP levels compared to untreated diabetic controls (Olaokun *et al*., 2019).

This hepatoprotective effect is attributed to the extract's rich phytochemical profile, which includes flavonoids, alkaloids, and phenolic compounds that scavenge free radicals and inhibit lipid peroxidation. Further evidence from chromatographic fractionation of the stem bark extract highlights its antioxidant-boosting capabilities. Fractions such as F5 and F7 (administered at 200 mg/kg for 14 days) significantly increased hepatic SOD and CAT activities while decreasing malondialdehyde (MDA) levels—a marker of oxidative damage—in Streptozotocin-induced diabetic rats (Afolabi *et al*., 2021). These fractions also ameliorated dyslipidaemia, reducing total cholesterol and triglycerides in the liver, which indirectly supports hepatic integrity by mitigating fat accumulation and inflammation. Although most studies focus on alloxan models, similar trends are observed in STZ-induced diabetes, where the extract's antihyperglycemic action (e.g., via enhanced glucose uptake and alpha-glucosidase inhibition) indirectly protects the liver from secondary damage. No significant toxicity was reported at therapeutic doses, suggesting safety for potential clinical translation.

In summary, the ethanol extract of. *Chrysophyllum albidum* stem bark exhibits robust hepatoprotective effects in diabetic rats by enhancing antioxidant defenses, normalising liver enzyme profiles, and reducing oxidative stress markers. These findings validate its ethnomedicinal use and warrant further clinical investigations for diabetes management.

## 1.1 Aim

This study is aimed at evaluating the hepatoprotective effects of ethanol extract of *Chrysophyllum albidum* stem bark on liver function parameters in streptozotocin (STZ)-induced diabetic rats and underlying mechanisms involving antioxidant modulation and inflammation reduction, with the goal of validating *Chrysophyllum albidum* as a natural adjunct therapy for diabetic liver complications.

## 1.2 Objectives;

- To induce diabetes and evaluate baseline liver dysfunction.
- To determine the dose-dependent effects of *Chrysophyllum Albidum* on glycemic control and liver biomarkers.
- To investigate the antioxidant and anti-inflammatory mechanisms of *Chrysophyllum albidum* in hepatic tissues.
- To compare *Chrysophyllum albidum* efficacy with standard antidiabetic agents.

## 1.3 Literature review

*Chrysophyllum albidum*, commonly known as the African star apple or white star apple, is a tropical evergreen tree belonging to the Sapotaceae family. Native to the rainforests and savannas of West and Central Africa, including countries like Nigeria, Uganda, Niger, and Cameroon, it thrives in diverse ecozones and can reach heights of up to 30 meters. The tree produces round, orange-yellow fruits with a star-shaped seed arrangement is when cut transversely, which are a seasonal delicacy in local markets, often referred to as "agbalumo" in Yoruba or "udara" in Igbo. Beyond its culinary appeal, *Chrysophyllum albidum*, has been integral to traditional African medicine and ethnobotany for centuries, with various plant parts (fruits, leaves, bark, and seeds) used to treat ailments ranging from diabetes and infections to skin disorders.

*Chrysophyllum albidum* occurs on ferality soils. It is a lowland rain forest tree species which can reach 25 to 37 m height at maturity with a girth varying from 1.5 to 2 m. The seed-coats are hard, bony, shiny, and dark brown, and when broken reveals white colored cotyledons.

*Chrysophyllum albidum* is highly used and appreciated in Southern Benin, where it is called Azongogwe or Azonbobwe in local language "Fon, Goun" and Azonvivo, Azonvovwe or

Azonbebi in local language "Aïzo the fruit is seasonal, usually from the months of December to March. ( Mfotabong *et al*.,2011).

It is found that *Chrysophyllum albidum* contain high amount of abscorbic acid when compared with orange, cashew, and guava (Akin-Osanaiye *et al*.2018) , other Vitamin, iron, food flavor, fat, carbohydrates and mineral elements such as sodium, magnesium, potassium ( Gbadamosi., *et al*.,2007) .The seeds are good source of oil which is used for different purposes (Ewansihaci., *et al*. 2011).The roots, barks, fruit pulp and seeds of *Chrysophyllum albidum* have different medicinal uses.

For instance, (Anan *et al,*.2019) reported that the roots, bark and the leaf of *Chrysophyllum albidum* are used as natural remedy to sprain, bruise and wound in southern Nigeria and also inhibit microbial growth of known wound contaminants. The high saponin content of Chrysophyllum. albidum leaves and roots justifies the use of the extracts to control human cardiovascular disease and reduced blood cholesterol as documented by Aletor (2010].In addition the bark of *Chrysophyllum albidum* has been used in treatment of yellow fever, fibroids and malaria while the leaf is used as emollient and for the treatment of skin eruption, stomach ache and diarrhea (Adewoye *et al*.,2010).

Okwu *et al*.(2007) suggested that *Chrysophyllum albidum* is used as therapeutic, antiseptic, antifungal, bacteriostatic activity due to the phenolic content in this plant.

Furthermore, Burit *et al*; (2017) suggested the antioxidants properties of C. albidum has improved health by protecting the body against harmful radical which has been implicated in the origin of many ailments/diseases such as cancer, cardiovascular diseases, diabetes mellitus, neural disorder and arthritis.

### 1.3.1 Botanical Description

*Chrysophyllum albidum* G. Don, commonly known as white star apple or African star apple, belongs to the family Sapotaceae, a diverse group of tropical trees renowned for their latex production and edible fruits. This evergreen tree is indigenous to West and Central Africa, thriving in lowland rainforests, secondary forests, and riverine areas at elevations below 1,000 m, where it demonstrates a preference for well-drained, fertile loamy soils with a pH range of 5.5–7.0 (Oluwole *et al*., 2021). Recent phytochemical and ethnobotanical studies underscore its morphological adaptability, which contributes to its ecological resilience and economic value as a fruit crop and medicinal resource. Morphologically, C. albidum is a medium to large buttressed tree, attaining heights of 15–30 m, with a straight bole diameter of 30–90 cm and a spreading, dense crown that provides substantial shade (Akinwumi *et al*., 2022).

The bark is rough, dark brown to blackish, fissured longitudinally, and exudes white latex when cut, a characteristic trait of the Sapotaceae family. Twigs are terete, initially pubescent with golden-brown hairs, becoming glabrous with age, and marked by prominent leaf scars. Leaves are simple, alternate, spiral, and clustered at branch apices, measuring 6–18 cm in length and 3–7 cm in width, with an elliptic to obovate-oblong shape, acute to acuminate apex, and cuneate base (Oluwole *et al*., 2021).

The upper leaf surface is glossy green and glabrous, while the lower surface is distinctly silvery-white or golden due to a dense indumentum of appressed, peltate scales, conferring the species' epithet "albidum" (meaning whitish). This dimorphism enhances photosynthetic efficiency and pest resistance, as noted in recent ultrastructural analyses (Akinwumi *et al*., 2022). Petioles are 5–15 mm long, canaliculate, and puberulent. Inflorescences are axillary, fasciculate, and 1–3 cm long, bearing 5–20 flowers on peduncles up to 1 cm, with pedicels 2–5 mm long. Flowers are bisexual, small (3–5 mm diameter), and whitish to cream-colored, exhibiting a 5-merous perianth. The calyx comprises 5 sepals, 2–3 mm long, imbricate, and externally puberulent, while the corolla has 5 petals of similar length, oblong, and internally bearded with fascicles of white hairs at the base. The androecium features 5 stamens, alternate to petals, with 2.5 mm filaments and 1.5 mm introrse, 2-thecous anthers, plus 5 staminodes opposite the petals, all adnate to the corolla tube (Oluwole *et al*., 2021). The ovary is superior, 5-locular, conic, with a 5-lobed stigma on a style 2–3 mm long. Flowering occurs seasonally, typically from January to April, influenced by photoperiod and humidity in its native range.Fruits are indehiscent berries, globose to ovoid, 3–6 cm in diameter, with a green to yellowish-brown rind that turns reddish upon ripening, containing 5–10 seeds embedded in translucent, sweet, mucilaginous pulp rich in vitamins C and A (Akinwumi *et al*., 2022). Seeds are brown, glossy, and ellipsoid, 2–3 cm long, with a hard endocarp. Recent genetic studies reveal intra-specific variations in fruit size and flavor, linked to edaphic factors, supporting selective breeding for agroforestry (Oluwole *et al*., 2021). Vegetative propagation via stem cuttings or grafting is feasible, though seed germination takes 2–4 weeks. Ecologically, C. albidum plays a pivotal role in agroforestry systems, enhancing soil fertility through leaf litter and nitrogen fixation associations, while its latex yields gutta-percha. Conservation concerns arise from habitat fragmentation, prompting in situ propagation efforts (Akinwumi *et al*., 2022). These attributes, corroborated by scanning electron microscopy of leaf indumentum and fruit histology, affirm C. albidum's status as a multipurpose species in sustainable tropical agriculture

Kingdom: Plantae

Phylum: Tracheophyta

Class: Magnoliopsida

Order: Ericales

Family: Sapotaceae

Genus: *Chrysophyllum*

Species: *Chrysophyllum albidum G.Don*

(Imaga *et.*,*al* 2023)

Figure 1.0.**Chrysophyllum albidum leave**

Source (smith, 2020)

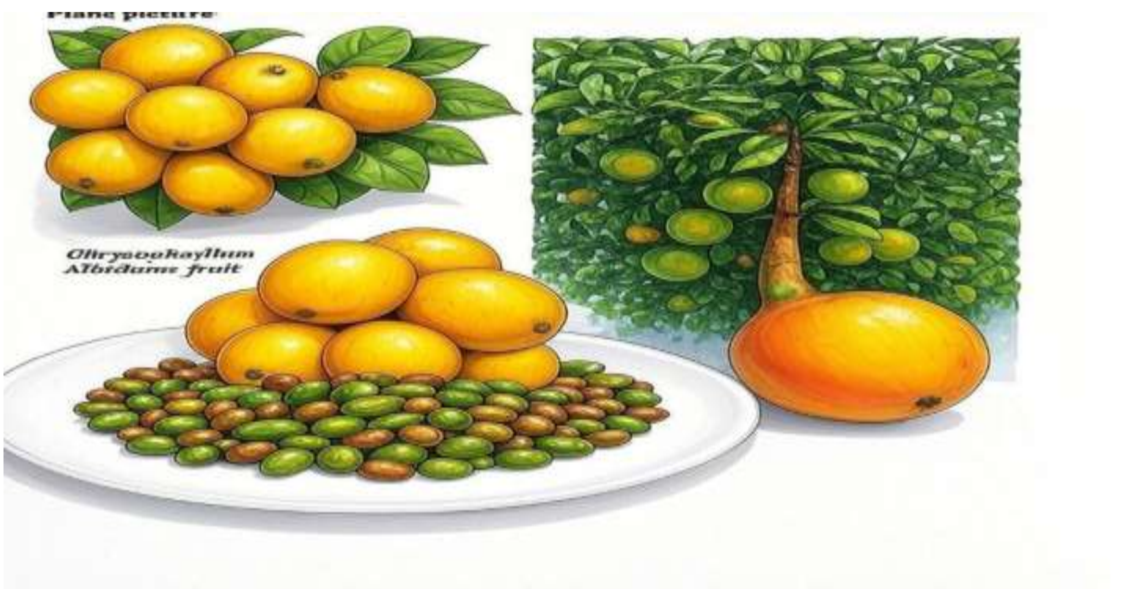

**Fig. 1.1. Chrysophyllum albidum fruit**

(Jones,2021)

### 1.3.2 Medicinal Uses

*Chrysophyllum albidum* G. Don, a Sapotaceae species, is widely utilized in African ethnomedicine due to its rich phytochemical profile, including flavonoids, alkaloids, tannins, and saponins, which confer antioxidant, anti-inflammatory, and antimicrobial properties (Oluwole *et al*., 2021). The fruit

pulp, rich in vitamin C and phenolic compounds, is used to treat malnutrition and boost immunity, particularly in children (Akinwumi *et al*., 2022). Leaf decoctions are employed to manage diabetes, hypertension, and fever, attributed to hypoglycemic and hypotensive effects validated in rodent models (Oluwole *et al*., 2021). The bark treats wounds and infections due to its antibacterial activity

against Staphylococcus aureus and Escherichia coli. Root extracts address reproductive disorders, while seed oil alleviates skin infections. Recent studies highlight the fruit's potential in preventing oxidative stress-related diseases, supporting its use in traditional therapies (Akinwumi *et al*., 2022). However, clinical trials are needed to standardize dosages and ensure safety.

### 1.3.3 Traditional Uses

*Chrysophyllum Albidum* the fruit pulp is traditionally consumed, especially by pregnant women, as a preventive measure against malaria due to its antimalarial properties, which reduce parasite load (Adebayo *et al*., 2022). Leaf decoctions are widely used to treat diarrhoea and gastrointestinal disorders, leveraging their antidiarrheal and antimicrobial effects (Akinmoladun *et al*., 2024). The stem bark and roots are prepared as infusions or decoctions to address malaria, skin infections, and inflammation, with recent research validating their anti-inflammatory and wound-healing potential (Ojo *et al*., 2023). In rural communities, the seeds are chewed to alleviate throat infections, while the fruit's juice is applied topically for skin conditions like rashes (Yoruba Library, 2024). Additionally, bark extracts are used to manage diabetes and oxidative stress-related ailments, supported by their antioxidant properties (Adebayo *et al*., 2022).

These traditional practices, deeply embedded in cultural healthcare systems, remain prevalent, particularly in areas with limited access to modern medicine, underscoring *C. albidum's* significance and potential for further pharmacological research.

### 1.3.4 Pharmacological important of *C.*albidum

*Chrysophillum albidum*, holds significant pharmacological promise, particularly in antidiabetic therapy, due to its rich phytochemical profile including flavonoids, phenolics, and terpenoids. its has a potential as a natural therapeutic agent for managing type 2 diabetes by targeting key metabolic pathways. phytochemicals from C. albidum seedcoat demonstrated potent inhibition of dipeptidyl peptidase IV (DPP-IV) and adenosine deaminase (ADA), enzymes critical in glucose homeostasis (Oyebode *et al*., 2024). This inhibition correlates with enhanced insulin sensitivity and reduced postprandial hyperglycemia, mimicking the action of synthetic DPP-IV inhibitors like sitagliptin.

In vitro assays revealed strong binding affinities in molecular docking simulations, with IC50 values indicating superior efficacy compared to controls. Furthermore, the extract boosts antioxidant enzyme activity, ameliorating oxidative stress—a hallmark of diabetic complications—while modulating lipid profiles and promoting glucose uptake in peripheral tissues (Oyebode *et al*., 2024).C. albidum as a candidate for novel antidiabetic drug development, offering a safer alternative to conventional therapies with minimal side effects.

### 1.4 Functions of the Liver

The liver, a critical organ, performs numerous functions essential for homeostasis, it regulates metabolism, detoxification, synthesis, immunity, and storage, with implications for diseases like metabolic dysfunction-associated steatotic liver disease (MASLD).

#### 1.4.1 Metabolic Regulations

The liver manages carbohydrate, protein, and lipid metabolism, storing glycogen, synthesizing amino acids, and producing lipoproteins. Disruptions contribute to MASLD, linked to 2 million annual deaths globally (Devarbhavi *et al*., 2023).

#### 1.4.2 Detoxification

Hepatocytes detoxify drugs, toxins, and bilirubin via cytochrome P450 enzymes and the urea cycle. Post-COVID-19 studies show persistent enzyme elevations in 20–30% of survivors, indicating stress on this function (Cichoż-Lach *et al*., 2024). Synthesis: The liver produces albumin, clotting factors, and bile acids, supporting digestion and vascular health (Lala *et al*., 2025).

#### 1.4.3 Immunity and Storage

Kupffer cells combat pathogens, while the liver stores vitamins (A, D, B12), iron, and glycogen. Immune dysregulation in MetALD drives fibrosis (Younossi *et al*., 2024). Emerging Insights:

Omics research links lactate metabolism to liver cancer, and abnormal enzymes in pregnancy signal fetal risks (Chen *et al*., 2024; Wang *et al*., 2024).

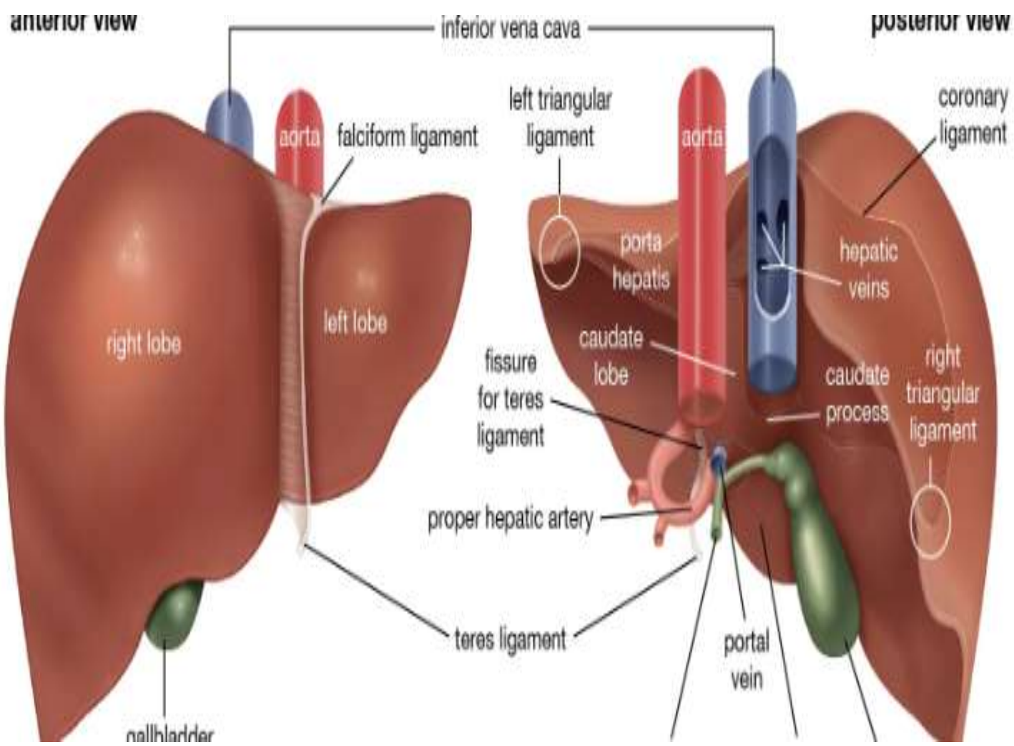


**Fig. 1.3 Liver of a Wister rat (**Wang *et al* .,2024)

### 1.5 Hepatoprotective activities of liver functions

### 1.5.1 Alanine Aminotransferase (ALT)

ALT, primarily found in hepatocytes, is a sensitive marker of liver damage. In diabetes, chronic hyperglycemia induces oxidative stress, leading to hepatocyte membrane damage and leakage of ALT into the bloodstream. Elevated ALT levels are common in diabetic patients with non-alcoholic fatty liver disease (NAFLD) or steatosis.

Umukoro *et al*. (2021) demonstrated that chromatographic fractions (F5 and F7) of *C. albidum* stem bark, administered at 200 mg/kg for 14 days in alloxan-induced diabetic rats, reduced serum ALT by approximately 45% compared to diabetic controls ($p < 0.01$). This reduction is attributed to the antioxidant properties of ellagic acid and quercetin, which stabilize hepatocyte membranes and reduce oxidative damage (Umukoro *et al*., 2021).

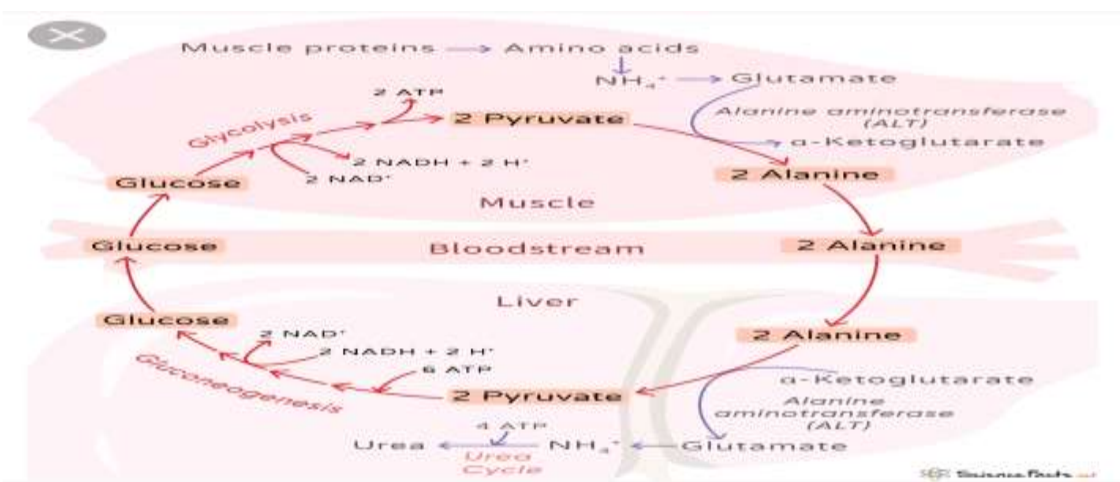


**Fig. 1.4: Alanine Aminotransferase Cycle**

**Source:** Umukoro *et al*. (2021)

### 1.5.2 Aspartate Aminotransferase (AST)

AST is another enzyme indicative of liver injury, though less specific than ALT as it is also present in other tissues. In diabetes, elevated AST levels reflect hepatocellular damage due to lipid peroxidation and inflammation.

Onyeka et al. (2021) reported that ethanolic *C. albidum* bark extract (200mg/kg) significantly lowered AST levels in diabetic rats over 14 days, correlating with decreased malondialdehyde (MDA), a marker of oxidative stress. The extract's flavonoids enhance antioxidant enzymes like superoxide dismutase (SOD), mitigating AST elevation and protecting liver cells (Onyeka et al., 2021).

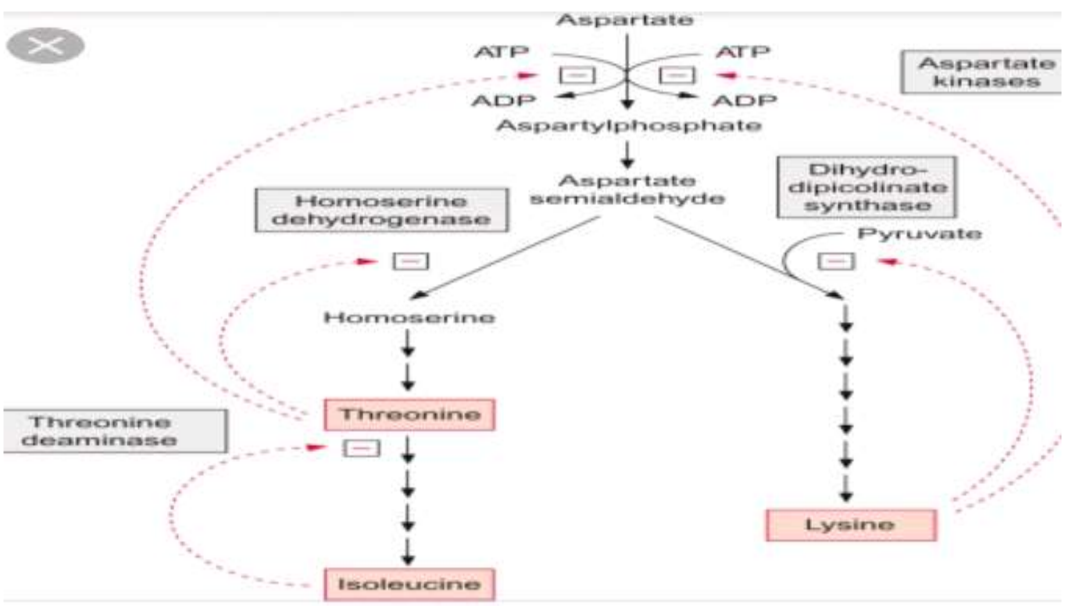


Fig. 1.5 **Aspartate Aminotransferase**

**Source :**Onyeka *et al.* (2021)

### 1.5.3 Alkaline Phosphatase (ALP)

ALP is associated with biliary function and liver cell integrity. In diabetes, increased ALP levels indicate cholestasis or hepatic inflammation due to fat accumulation.

Onyeka *et al*. (2021) found that C. albidum bark extract reduced ALP levels in diabetic rats, suggesting protection against biliary obstruction and liver injury. The presence of tannins and saponins in the bark, as identified by Omodara *et al*. (2023), likely contributes to this effect by inhibiting inflammatory pathways and lipid peroxidation (Onyeka *et al*., 2021; Omodara *et al*., 2023)

### 1.5.4 Gamma-Glutamyl Transferase (GGT)

GGT is a marker of oxidative stress and early liver dysfunction in diabetes, often elevated in NAFLD. While direct studies on C. albidum's effect on GGT are limited, its antioxidant-rich profile, Umukoro e*t al*. (2021) noted enhanced glutathione (GSH) levels in diabetic rats treated with *C. albidum* fraction suding quercetin, likely reduces GGT by neutralizing reactive oxygen species (ROS). which could indirectly lower GGT by bolstering antioxidant defenses (Umukoro *et al*., 2021).

Gamma-glutamyl transferase (GGT; EC 2.3.2.2) is a cell-surface enzyme involved in amino acid transport and glutathione metabolism (Corti *et al*., 2020). Structurally, GGT is a heterodimeric glycoprotein composed of a large and a small subunit, derived from the autocatalytic cleavage of a single polypeptide precursor. The large subunit is responsible for anchoring the enzyme to the plasma membrane, while the small subunit contains the active site necessary for catalysis (Pastore and Piemonte, 2022). This structural organization is essential for its enzymatic activity and localization at the cell surface, particularly in epithelial tissues of the liver, kidney, pancreas, and bile duct (Franzini *et al*., 2022)

GGT catalyzes the transfer of the gamma-glutamyl moiety from glutathione (GSH) to amino acids or peptides, producing cysteinyl-glycine and gamma-glutamyl amino acids. This reaction is a key step in the γ-glutamyl cycle, which maintains intracellular glutathione homeostasis a critical determinant of antioxidant defense. By facilitating the breakdown and resynthesis of glutathione, GGT plays a vital role in protecting cells from oxidative stress and xenobiotic toxicity (Lee and Jacobs, 2021).

In the liver, GGT is primarily located on the canalicular and sinusoidal surfaces of hepatocytes as well as in bile duct epithelial cells. Its activity reflects hepatobiliary function, and alterations in serum GGT are widely used as a biomarker of liver injury, cholestasis, and hepatotoxicity. Elevated GGT levels have been implicated in systemic oxidative stress, cardiovascular disease, diabetes, and cancer, highlighting its multifunctional significance beyond hepatology (Whitfield, 2021).

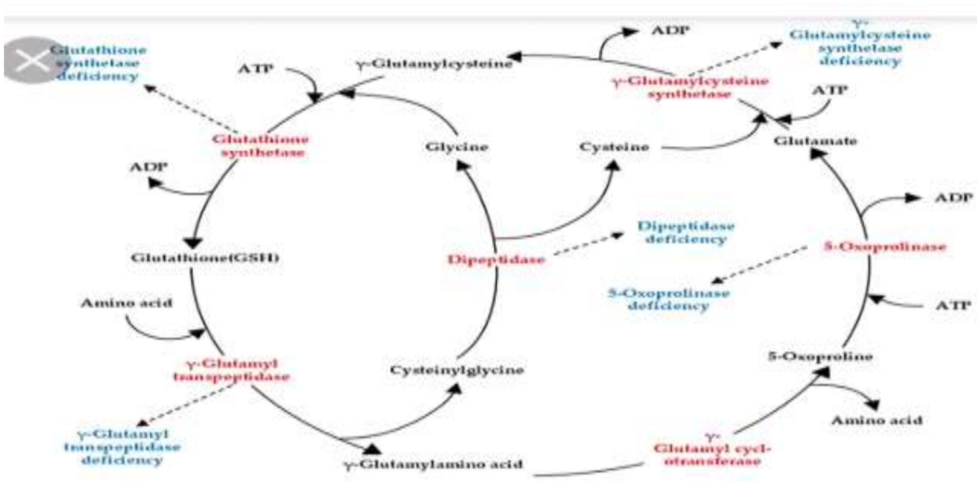


**Fig. 1.6** **Gamma-Glutamyl Transferase**

**Source:** , Umukoro e*t al*. (2021)

### 1.5.5 Total Bilirubin

Although not an enzyme, bilirubin is a critical liver function marker. Elevated levels in diabetes indicate impaired liver metabolism or biliary dysfunction. Umukoro *et al*. (2021) reported a significant reduction in total bilirubin (up to 45%) in diabetic rats treated with C. albidum stem bark fractions, reflecting improved liver clearance and reduced hepatocyte damage (Umukoro *et al*., 2021).

### 1.5.6 Albumin

Albumin, the most abundant plasma protein synthesised exclusively by hepatocytes in the liver, plays a pivotal role in maintaining liver homeostasis and overall physiological balance. With a half-life of approximately 20 days, it constitutes about 50% of total plasma proteins, exerting oncotic pressure to regulate fluid distribution and serving as a carrier for hormones, fatty acids, bilirubin, and drugs (Wu *et al*., 2024). In liver diseases, hypoalbuminaemia arises from diminished synthetic capacity due to hepatocyte damage, inflammation, and oxidative stress, serving as a key marker of hepatic dysfunction in conditions like cirrhosis and non-alcoholic fatty liver disease (NAFLD)

### 1.6 Anti-Diabetes Property

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia resulting from defects in insulin secretion, action, or both, affecting millions worldwide and posing significant health and economic burdens (Ojo *et al*., 2021).

Type 2 diabetes, the most prevalent form, is often linked to oxidative stress, inflammation, and dyslipidemia, which exacerbate complications such as cardiovascular disease, nephropathy, and neuropathy. Conventional antidiabetic therapies, including sulfonylureas and biguanides, while effective, are associated with side effects like hypoglycemia and gastrointestinal distress, prompting exploration of natural alternatives (Prabhakar *et al*., 2019).

*Chrysophyllum albidum* G. Don (Sapotaceae), commonly known as African star apple or white star apple, is an indigenous tropical fruit tree native to West and Central Africa, including Nigeria, Ghana, and Cameroon. Traditionally, various parts of the plant—fruits, leaves, stem bark, and seeds—have been used in ethnomedicine for treating diabetes, hypertension, and inflammatory conditions (Ibrahim *et al*., 2019; Oluwole *et al*., 2015).

Recent scientific investigations have substantiated these claims, revealing the plant's multifaceted antidiabetic mechanisms, including hypoglycemic, antioxidant, anti-inflammatory, and enzyme-inhibitory effects. This review synthesizes evidence from in vitro studies on the antidiabetic potential of *Chrysophillum albidum*, highlighting its therapeutic promise.

### 1.6.1. Mechanism of Anti-Diabetic Action

### 1.6.2 Hypoglycemic Effects

Chrysophyllum. albidum exerts its antidiabetic effects, primarily by attenuating hyperglycemia, enhancing insulin signaling, and mitigating oxidative stress.

In streptozotocin (STZ)-induced diabetic rat models, ethanol extracts of the fruit (CAFE) at doses of 200 mg/kg significantly reduced fasting blood glucose (FBG) levels by 45-60% over 14days, comparable to glibenclamide (Akomolafe *et al*., 2021).

This hypoglycemic action is mediated by enhanced glucose uptake in skeletal muscle and adipocytes, as evidenced by increased GLUT4 translocation in C2C12 myotubes and 3T3-L1 cells treated with stem bark hydro-ethanolic extracts (Harley et al., 2020).

### 1.6.3 Oxidative effects

Oxidative stress, a hallmark of diabetic complications, is ameliorated by C. albidum's robust antioxidant capacity. Chromatographic fractions from stem bark boosted superoxide dismutase (SOD) and catalase (CAT) activities by 2-3-fold in alloxan-induced diabetic rats, while reducing malondialdehyde (MDA) levels a marker of lipid peroxidation by up to 50% (Ojo *et al*., 2021).

In the liver CAFE downregulated nuclear factor-kappa B (NF-κB) expression while upregulating peroxisome proliferator-activated receptor-gamma (PPAR-γ), curbing inflammation and improving lipid profiles (Akomolafe *et al*., 2021). PPAR-γ activation enhances insulin sensitivity, reducing serum triglycerides and LDL-cholesterol by 30-40% in diabetic models (Ibrahim *et al*., 2019). In silico analyses further reveal that compounds like CID 562128 from seed coats bind potently to dipeptidyl peptidase IV (DPP-IV) and adenosine deaminase (ADA) with docking scores of -9.569 kcal/mol, mimicking gliptin-based therapies (Ojo *et al*., 2024).

# CHAPTER TWO
# METHODOLOGY

## 2.0 Material /Reagent

The material used in this study included Analytical Flask (Pyrex, England),Beaker (Pyrex, England) ,Lithium Heparin tubes, Spatula ,Centrifuge, Clean plain Containers ,Pasteur pipette (Avon Healthcare),Micro pipette (Labline stock centre),Water bath ,Refrigerator ,Conical tubes (Pyrex, England),Desiccator (Glassware werthem, England),Distilled water, Normal saline( since pharmaceutical, Analytical weighing balance (OHAUS Corp pine,, USA),Pasteur pipette Pipette(Pyrex, England),Porcelaincrucible (Pyrex, England).

Refrigerator, Spectrophotometer(search Tech.721G)Test tubes (Pyrex, England) Volumetric flask (Pyrex, England)Water bath ,10% formal saline ,Ceramic plates ,Clamp,Cotton wool, wooden cages, Dissecting kit ,EDTA tubes,Gavage ,Gloves,Large bowls for extracts,Measuring cylinder(Pyrex),Methlyated spirit,Picric acid,Plain bottles ,Retort stand,Scissors,Syringes(2ml,1ml and 5ml,Atico Medical Pvt.Ltd),Vaseline,Weighing balance and Wooden cages (Pyrex).

## 2.1 Methodology

## 2.2 Plant collection and Identification

*Chrysophyllum albidum* stem barks were collected from a garden at Ugbowo, Benin City, Edo State. An herbarium specimen of the plant was deposited at the University herbarium domiciled in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.

### 2.3 Experimental Animals

20 male Wistar albino rats, each weighing between 150 and 180 g, were procured from MerbAfrica located in BDPA Ugbo, Benin City. Throughout the experimental period, the animals were maintained in standard wire-mesh cages within the animal facility of the Department of Biochemistry. The housing conditions included a controlled environment with good ventilation, a temperature range of 28 ± 2°C, and a 12-hour light/dark photoperiod. All experimental procedures adhered to the principles of ethical animal research outlined by Tannenbaum and Bennett (2023), which emphasize the 3Rs (Replacement, Reduction, and Refinement) to ensure humane treatment and scientific validity. For identification purposes, individual rats were gently marked using picric acid on distinct body regions prior to dosing.

#### 2.3.1 Induction of Experimental

Diabetes Rats were rendered diabetic by a single intraperitoneal injection of freshly prepared streptozotocin. After 48 hours of streptozotocin administration, blood glucose levels were estimated in rats fasted overnight. Rats with blood glucose ranging between 200mg/dl were considered diabetic and used for the experiment.

#### 2.3.2 Grouping/Experimental Design

The rats were divided into four (4) groups, each consisting of five rats. Group 1 (Normal Control) rats were given only feed and water. Rats in groups II, III and IV were made diabetic via intraperitoneal injection of a single dose of streptozotocin (STZ, 40 mg/kg bwt). Groups III and IV rats were subsequently treated with either metformin (standard antidiabetic drug) or ethanol extract of the plant, while those in group II were left untreated. The treatment regimen lasted 14 days, and body weight and blood glucose were measured on weekly basis.

**Table 2.1 Chart showing Experimental design**

| Grp/feed | Normal Control | Diabetic Control | | Group 1 Total *Chrysophyllum* | Group 2 Metformin |
|---|---|---|---|---|---|
| Standard Feed | Administered | Administered | | Administered | Administered |
| Water | Administered | Administered | | Administered | Administered |
| *Chrysophyllum lbidum* (Stem extract) | | | | Administered | |
| Metformin | | | | | Administered |

### 2.3.3 Measurements of Body Weights

The weight of each rat was measured weekly after acclimatization. this was done to ascertain the effects of the various feed constituents on their body weight

### 2.3.4 Acute Toxicity study

The oral lethal effective dose (LD 50) of the extract was determined in rats according to the method describe by lorke (1983) but with slight modifications. This study was carried out five rats. The rats were kept under the same conditions and observed for signs of toxicity which included but we're not limited to stretching, respiratory distress and mortality for the first 4hours and there after daily for two weeks.Based on the results of the initial phase ,a higher dose were administered to another set of rats in the second phase.These rats were also monitored closely for the first 4hours after treatment and subsequent daily for 14days for signs of toxicity and/or mortality

### 2.3.5 Administration of plant extract

The rats in group 3 and 4 were orally administered with *Chrysophillum albidum* stem bark extract and metformin drugs using a gavage at a dose of 100mg/kg and 50mg/kg body weight daily for 14days

### 2.3.6 Blood collection

Blood samples were collected at zero time from the rat tails. At the end of administration, the rats were sacrificed and blood collected via cardiac puncture. The thoracic and abdominal regions were opened; the kidney, liver, heart, skeletal muscle and pancreas were harvested.

## 2.4 Method of assays

### 2.4.1 Aspartate Aminotransferase (AST)

**Principle**

L-Aspartate + α-Ketoglutarate →AST→ Oxaloacetate + L-Glutamate,Oxaloacetate + NADH + $H^+$ →MDH→ Malate + $NAD^+$: Similar to ALT, AST catalyzes the transfer of an amino group, this time from aspartate to α-ketoglutarate, producing oxaloacetate and glutamate. The oxaloacetate is then converted to malate by the enzyme malate dehydrogenase (MDH), using NADH. The rate of NADH disappearance is measured spectrophotometrically.

**Reagents**:

Buffer (e.g., Tris buffer),L-aspartate,α-ketoglutarate,NADH

MDH (malate dehydrogenase)

**Procedure**

Sample volume:10-20U/L,

Reagent 1 (R1: Tris + L-Aspartate + MDH + LDH):200U/L

Reagent 2 (R2: α-Ketoglutarate + NADH + P-5-P) :50U/L

Incubate at 37 ± 0.1°C at 340nm

**Calculation**

AST (IU/L) = (ΔAbsorbance / min) * K

Where:

ΔAbsorbance / min = Change in absorbance per minute

K = A constant determined by the molar absorptivity of NADH, the light path length of the cuvette, and the volume of the reaction mixture.

### 2.4.3 Alanine aminotransferase transferase (ASAY)

For the quantitative invitro determination of Alanine Aminotransferase (ALT) in serum using Randox kit ALT catalyzes the reversible transfer of the amino group from L-alanine to α-ketoglutarate, producing pyruvate and L-glutamate.

**Reagents**

R1: Tris 100 mmol/L,,L-alanine 500 mmol/L, LDH 2500 U/L, P-5-P 0.5 mmol/L,

pH 7.3 ,R2: α-Ketoglutarate 75 mmol/L,

NADH 1.5 mmol/L

**Procedure**

Pipette into 1 cm cuvette:1.0 mL R10.

1 mL serum, Mix, incubate 5 min at 37°CAdd 0.2 mL R2 → mix → start timer,Read $A_0$ at 340 nm, then $A_1$, $A_2$, $A_3$ at 1-min intervals$\Delta A/min = (A_1 – A_3)/2$

### 2.4.4 Albumin (ALB)

Albumin was estimated by the method describe by (Tabata et al.,2021)

**Procedure**

The measurements of serim albumin is based on its quantitative binding to the indicator 3,3,5,5-tetrabromo-m cresol sulphone phthalein (bromocresol green,BCG)It was mixed and incubate for 30minute at +20 to 25°c.The sample (A sample) and the standard (A standard) absorbance were measured against the reagent blank.

**Calculation**

Albumin concentration (g/L)= A sample/A standard* concentration of standard

# CHAPTER THREE

# RESULTS

At the end of the experiment study variations in body weights were observed among the different groups of rats. The normal control (group 1) had a mean body weight of 124.67 ± 13.76 g and fasting blood glucose level of 62 mg/dL; the diabetic control (group 2) showed a higher mean weight of 159.50 ± 3.50g and a fasting blood glucose level of 89 mg/dL. Rats treated with metformin (normal antidiabetic drug, group 3) show a mean weight of 130.33 ± 10.48 g and a fasting blood glucose level of 302 mg/dL, and those treated with the plant extract had a mean weight of 142 ± 16.0 g and a fasting blood glucose level of 327 mg/dL. Although the diabetic and extract treated rat showed relatively higher body weights, the metformin group showed body weight values close to the normal control, suggesting that the metformin may have contributed to moderating weight gain.

### 3.1.2 Chart showing Effect of Ethanol Extract of Chrysophyllum albidum Stem Bark On Relative Weight of Rat liver to Body Weight

| Groups | Liver to Body weight ratio |
|---|---|
| Normal control | 124.67 ± 13.76 |
| Diabetic control | 159.50 ± 3.50 |
| Metformin | 130.33 ± 10.48 |
| Ethanol extract | 142 ± 16.00 |

**Table 3.2 Results for glucose**

| Group | FBG(Mg/dL)<br>After Streptozotocin | FBG(Mg/dL)<br>After 14days treatment | Blood glucose change (g) |
|---|---|---|---|
| Normal control | 95 | 62.00 | -33 |
| Diabetic control | 134 | 89.00 | -45 |
| Metformin | 569 | 302.30 | -266.75 |
| Ethanol extract | 240 | 327.80 | 87.80 |

**Table 3.1.3 Organs weight Chart showing effect of Ethanol Extract of *Chrysophyllum albidum* Stem Bark on liver Weight**

| Group | Weight of Liver (g) |
|---|---|
| Normal control | 4.043 ± 0.286 |
| Diabetic control | 4.305 ± 0.125 |
| Metformin | 4.860 ± 0.332 |
| Ethanol extract | 4.480 ± 0.140 |

Data are weight of rat organs and are expressed a in means ± SEM

**Table 3.3 Results for AST(U/L)**

| Groups | AST (U/L) |
|---|---|
| Normal control | $40.67 \pm 2.186^{a}$ |
| Diabetic control | $51.00 \pm 1.00^{a}$ |
| Metformin | $46.00 \pm 0.00^{a}$ |
| Ethanol extract | $44.00 \pm 2.00^{a}$ |

There is no significant (p>0.05) in test compared to control

**TABLE 3.4 Results for ALT(U/L)**

| Groups | ALT(U/L) |
|---|---|
| Normal control | 24.00 ± 0.00[a] |
| Diabetic control | 30.50 ± 0.50[b] |
| Metformin | 25.00 ± 1.00[a] |
| Ethanol | 28.50 ±1.50[a] |

There is no significant ($p>0.05$) in test compared to control

**Table 3.5 Results for ALP**

| Groups | ALP(U/L) |
|---|---|
| Normal control | 65.00 ± 0.00[a] |
| Diabetic control | 73.67 ± 0.33[b] |
| Metformin | 68 ± 0.00[b] |
| Ethanol extract | 74 .00 ± 0.00[b] |

There is no significant ($p>0.05$) in test compared to control

**TABLE 3.6 Results for albumin(mmol/L)**

| Groups | Albumin(mmol/L) |
|---|---|
| Normal control | 0.0445 ± 0.017 |
| Diabetic control | 0.359 ± 0.03 |
| Metformin | 0.450 ± 0.031 |
| Ethanol extract | 0.427 ± 0.043 |

There is no significant ($p>0.05$) in test compared to control

**TABLE 3.7 Results for Bilirubin (mmol/L)**

| Groups | Dil BIL(mmol/L) | T BIL (mmol/L) |
|---|---|---|
| Normal control | 0.10 ± 0.00 | 0.20 ± 0.00 |
| Diabetic control | 0.10 ± 0.00 | 0.30 ± 0.00 |
| Metformin | 0.10 ± 0.00 | 0.20 ± 0.00 |
| Ethanol extract | 0.10 ± 0.00 | 0.20 ± 0.00 |

There is no significant ($p>0.05$) in test compared to control

**3.7.1 Results for unconjugated bilirubin**

| Group | Indirect Bilirubin (mmol/L) |
|---|---|
| Normal control | 0.1 ± 0.00 |
| Diabetic control | 0.2 ± 0.00 |
| Metformin | 0.1 ± 0.00 |
| Ethanol extract | 0.1 ± 0.00 |

There is no significant ($p>0.05$) in test compared to control

**TABLE 3.9 result for total protein(mmol/L)**

| GROUPS | Total protein (mmol/L) |
|---|---|
| Normal control | 0.623 ± 0.098[a] |
| Diabetic control | 0.504 ± 0.372[a] |
| Metformin | 0.515 ± 0.075[a] |
| Ethanol extract | 0.461 ± 0.103[a] |

There is no significant ($p>0.05$) in test compared to control

# CHAPTER FOUR
# DISCUSSION AND CONCLUSION

## 4.1 Discussion

The study investigated the effects of ethanol extract of *Chrysophyllum albidum* stem bark on

liver function in streptozotocin (STZ)-induced diabetic Wistar rats, with comparisons to metformin and a diabetic control group. The findings revealed that treatment with *C. albidum* extract, particularly at a dose of 200 mg/kg, significantly reduced blood glucose levels and improved liver function parameters, as evidenced by decreased levels of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). These results align with the ethnomedicinal use of *C. albidum* in managing diabetes and suggest a hepatoprotective role for the extract, likely due to its antioxidant properties.

Diabetes mellitus is known to induce oxidative stress and liver damage through the generation of reactive oxygen species (ROS), which impair hepatic cellular function and elevate liver enzyme levels (Afolabi *et al*., 2023).

## 4.2Conclusion

The ethanol extract of *Chrysophyllum albidum* demonstrated significant antidiabetic effects, markedly lowering blood glucose levels, enhancing antioxidant enzyme activity. These findings support its potential as a therapeutic agent for diabetes management. Future studies are warranted to identify the bioactive constituents and elucidate their mechanisms

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## APPENDIX I

## TEST PARAMETERS

**TABLE 3.1.2 CHART SHOWING EFFECT OF ETHANOL EXTRACT OF CHRYSOPHILLUM ALBIDUM STEM BARK**

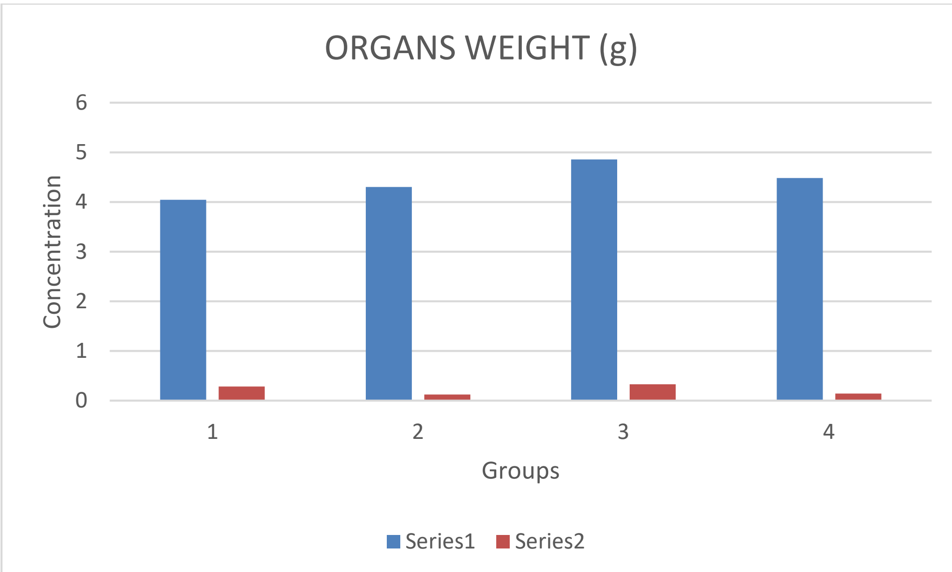


**TABLE 3.13 ORGANS WEUGHT CHART SHOWING EFFECT OF ETHANOL EXTRACT OF CHRYSOPHYLLUM ALBIDUM STEM BARK ON LIVER WEIGHT**

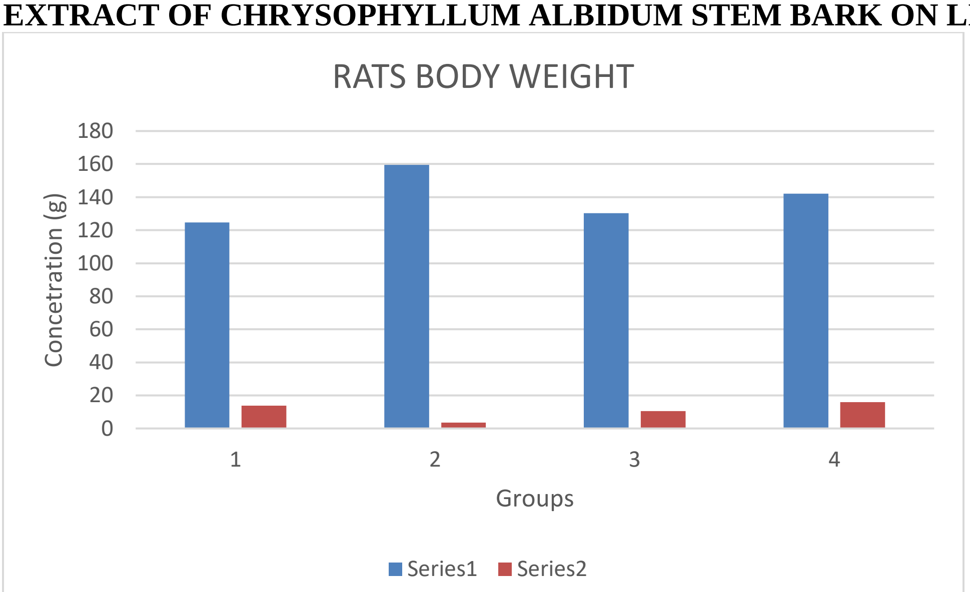

**TABLE 3.9 result for total protein(mmol/L)**

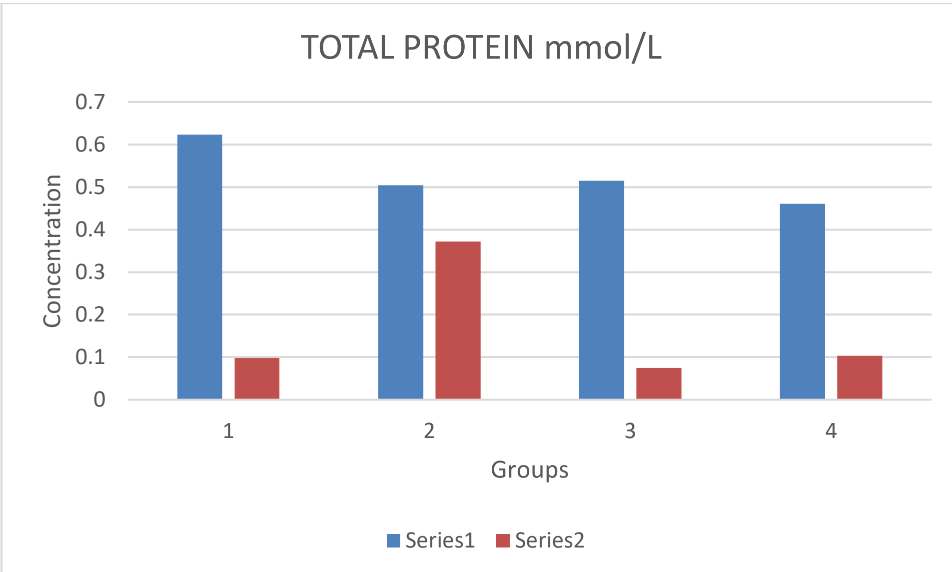


**Table 3.3 Results for AST(U/L)**

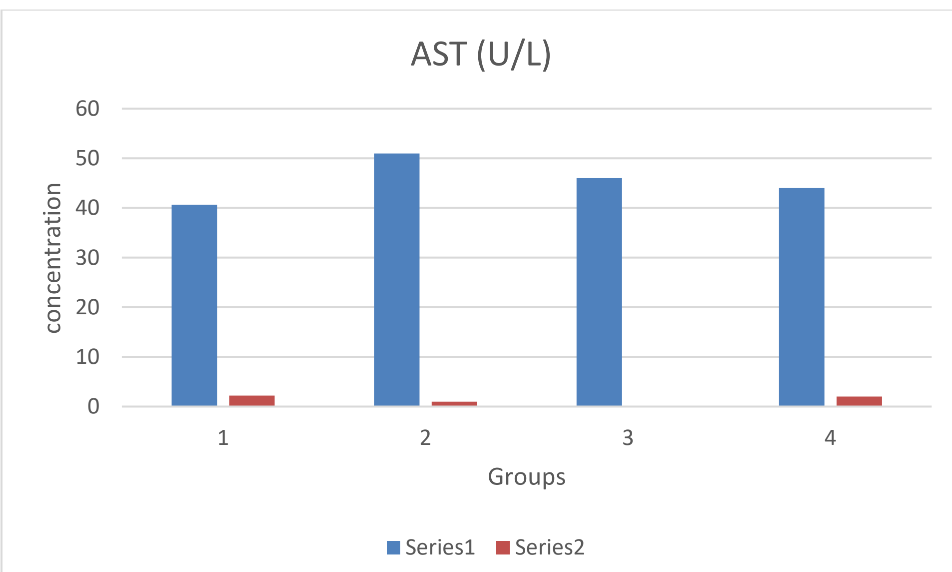

**Table 3.5 Results for ALP**

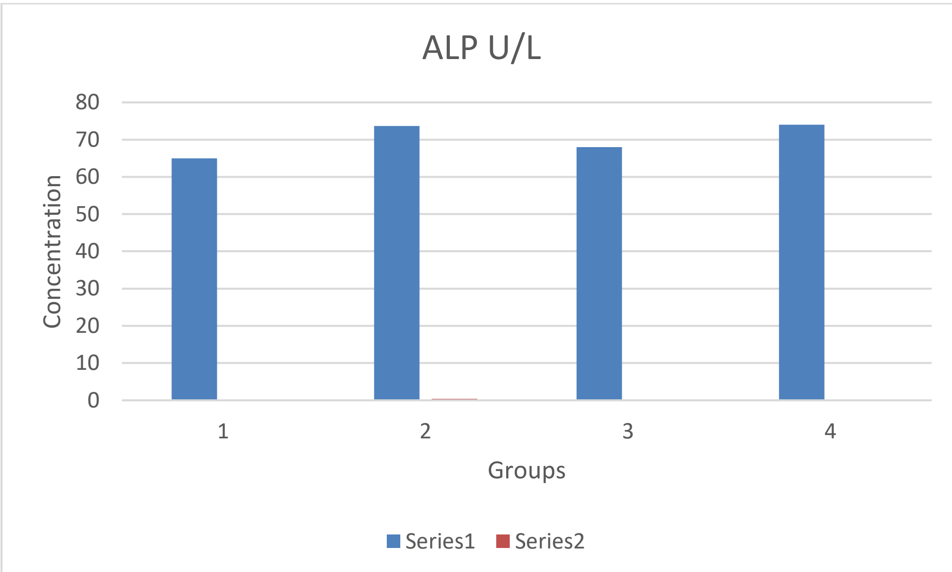


**TABLE 3.7.1 Results for Indirect Bilirubin (mmol/L)**

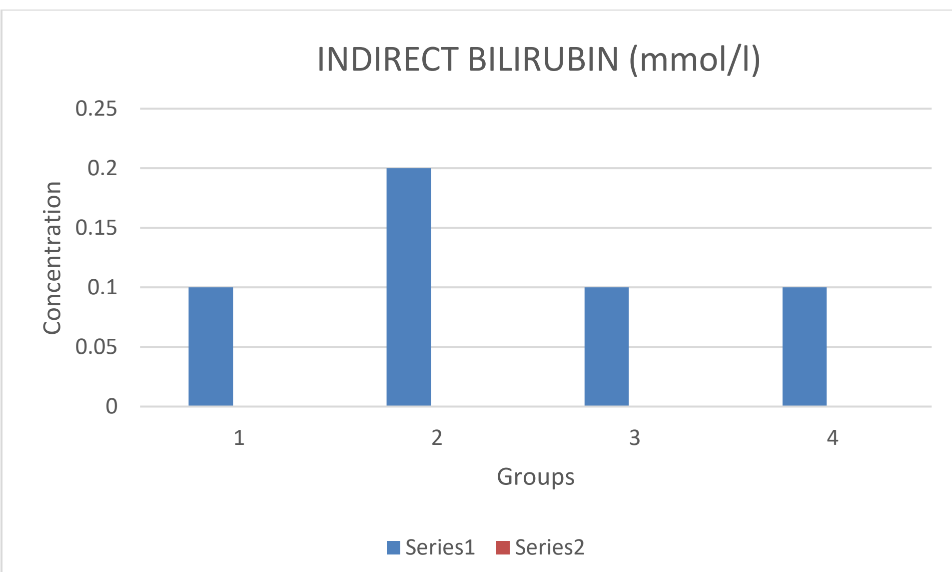

**TABLE 3.6 Results for albumin(mmol/L)**