The Influence of Picralima nitida on Glycated Hemoglobin and Serum Phosphate Concentrations in male Wistar Rats.

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BY

DR. Stephen Ewemade OGHAGBON

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CERTIFICATION

We the undersigned hereby certify that Benedicta Adesuwa OMOREGBE carried out this research in the Department of Medical Biochemistry, University of Benin, Benin City and thereby approve same as adequate in scope and quality for the award of Bachelor of Science Degree (B.Sc) in Medical Biochemistry. Signed:

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ABSRTRACT

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia, which often leads to complications such as increased glycated hemoglobin (HbA1c) levels and imbalances in serum phosphate concentrations. Picralima nitida, a medicinal plant widely used in traditional medicine, has shown potential antidiabetic and biochemical regulatory effects. This study aimed to evaluate the influence of P. nitida on glycated hemoglobin and serum phosphate concentrations in male Wistar rats after a controlled feeding period.

A total of [40] male Wistar rats were divided into experimental groups, including a control group and treatment groups receiving varying doses of P. nitida extract. Blood samples were collected before and after the intervention to assess HbA1c levels and serum phosphate concentrations. The results revealed a significant reduction in HbA1c levels in treated groups compared to the control, indicating improved glycemic control. Additionally, P. nitida administration didn't influenced serum phosphate concentrations.

These findings highlight the therapeutic potential of Picralima nitida in diabetes management and its possible impact on phosphate metabolism. Further studies are recommended to explore the underlying mechanisms and confirm its clinical applicability.

CHAPTER 1

Background of the study

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia due to insufficient insulin production, insulin resistance, or both. One of the major complications of diabetes is its effect on carbohydrate, protein, and mineral metabolism, leading to alterations in biochemical markers such as glycated hemoglobin (HbA1c) and serum phosphate levels (American Diabetes Association, 2022). Glycated hemoglobin is widely used as an indicator of long-term glycemic control, while serum phosphate plays a crucial role in energy metabolism, cell signaling, and bone health (Pal et al., 2018).Herbal medicine has gained considerable attention as an alternative or complementary therapy for managing diabetes due to its potential efficacy and minimal side effects compared to conventional drugs (World Health Organization, 2019). *Picralima nitida*, commonly known as the "Akuamma plant," is a medicinal plant found in West and Central Africa. It has been traditionally used for treating various ailments, including fever, pain, and diabetes (Ogbunugafor et al., 2011). However, limited scientific studies have investigated its effect on key metabolic biomarkers like glycated hemoglobin and serum phosphate concentrations. Glycated hemoglobin (HbA1c) is a form of hemoglobin that is chemically bound to glucose. It reflects the average blood glucose levels over a period of 8–12 weeks and is a crucial parameter in the diagnosis and management of diabetes (Nathan et al., 2007). Studies have shown that reducing HbA1c levels significantly lowers the risk of diabetes-related complications such as neuropathy, nephropathy, and cardiovascular diseases (UK Prospective Diabetes Study Group, 1998).Several medicinal plants have been reported to lower HbA1c levels through mechanisms such as enhancing insulin secretion, improving glucose uptake, and reducing oxidative stress (Patel et al., 2021). *Picralima nitida* contains bioactive alkaloids such as akuammine and akuammidine, which are believed to exhibit hypoglycemic properties (Akinmoladun et al., 2020). Investigating its effect on HbA1c will provide further insights into its potential as an antidiabetic agent.

Serum Phosphate and Its Metabolic Significance

Phosphate is an essential mineral involved in energy metabolism, bone formation, and cellular signaling (Berndt et al., 2007). In diabetic patients, disturbances in phosphate homeostasis have been observed, with some studies linking hypophosphatemia to poor glycemic control and increased insulin resistance (Faridi et al., 2021).

Several reports suggest that alterations in phosphate levels can contribute to diabetic complications, particularly in patients with chronic kidney disease or cardiovascular diseases (Eddington et al., 2013). Herbal therapies with potential glucose-lowering effects may also influence phosphate metabolism. Therefore, it is necessary to explore whether *Picralima nitida* affects serum phosphate concentrations, which could have broader implications for diabetic care.

Pharmacological Properties of *Picralima nitida*

Picralima nitida is a tropical medicinal plant belonging to the Apocynaceae family. It is rich in alkaloids, flavonoids, and other bioactive compounds known for their antimicrobial, analgesic, anti-inflammatory, and potential antidiabetic properties. The plant's seeds, bark, and leaves have been used in traditional African medicine for treating malaria, pain, and metabolic disorders (Aj ahassan et al., 2017).

Recent pharmacological studies suggest that extracts of *Picralima nitida* exhibit antioxidant and anti-inflammatory properties, which may contribute to its therapeutic potential in metabolic diseases . However, limited research has focused on its specific effects on glycated hemoglobin and serum phosphate levels. This study aims to bridge this gap by investigating its metabolic influence in Wistar rats under a controlled feeding interval.

1.2 Aim of research

The primary aim of this research is to analyze the influence of *Picralima nitida* on glycated hemoglobin (HbA1c) levels and serum phosphate concentrations in Wistar rats following a supervised feeding interval. This study seeks to investigate whether *Picralima nitida*, a medicinal plant traditionally used for diabetes management, has significant effects on long-term glucose control and phosphate metabolism, which are crucial biochemical parameters in diabetic pathophysiology.

Specific Objectives

To achieve the primary aim, the study is designed to:

Evaluate the effect of *Picralima nitida* on glycated hemoglobin (HbA1c) levels in Wistar rats to determine its potential role in long-term blood glucose regulation.Assess the impact of *Picralima nitida* on serum phosphate concentrations to understand whether it influences phosphate homeostasis, which is essential for metabolic processes and diabetic complications.
Determine the dose-dependent response of *Picralima nitida* administration by evaluating different concentrations to identify the optimal dosage for metabolic benefits.
Investigate potential correlations between changes in glycated hemoglobin and serum phosphate levels to explore possible interactions between glucose metabolism and phosphate balance.
Examine any adverse effects associated with the administration of *Picralima nitida* over the supervised feeding interval to assess its safety profile for future therapeutic applications.

1.3 Scope of the Study

This study focuses on evaluating the influence of *Picralima nitida* on glycated hemoglobin (HbA1c) levels and serum phosphate concentrations in Wistar rats following a supervised feeding interval. The research is designed to provide scientific insights into the metabolic effects of *Picralima nitida*, particularly about its potential for diabetes management and phosphate metabolism regulation.
The experimental subjects for this study are Male Wistar rats, a widely used animal model in biomedical research due to their genetic stability, physiological similarity to humans in metabolic studies, and suitability for controlled feeding experiments.
The study will be conducted in a controlled laboratory environment, where the Wistar rats will be housed under standard conditions of temperature, humidity, and light-dark cycles. The laboratory setting will allow for precise monitoring of dietary intake, controlled administration of *Picralima nitida* extract, and accurate measurement of biochemical parameters.
The study will be carried out over a supervised feeding interval, spanning a period sufficient to observe changes in glycated hemoglobin and serum phosphate levels. The exact duration will be based on standard metabolic response times in Wistar rats, typically ranging from four to eight weeks, to ensure adequate exposure to *Picralima nitida* and measurable physiological changes.
While this study aims to provide valuable insights into the metabolic effects of *Picralima nitida*, it is limited to: Animal models only: The findings may not be directly extrapolated to humans without further clinical trials.

Two metabolic markers (HbA1c and phosphate): Other potential biochemical effects of *Picralima nitida*, such as lipid profiles, renal function, and oxidative stress markers, will not be covered in this study.

1.4 Significance of the Study

Understanding the interaction between Glycated hemoglobin and phosphate glucose levels can cause serious clinical results in the treatment of diabetes.

If there is a strong correlation, serum phosphate may serve as an additional marker of glucose control and help to better reduce the risk of diabetic complications. Furthermore, identifying phosphate abnormalities in diabetic patients could facilitate early intervention strategies to prevent metabolic and cardiovascular complications.

Studying this relationship may explain pathophysiological mechanisms linking diabetes, phosphate homeostasis, and renal function. This knowledge may facilitate the development of novel therapeutic approaches targeting phosphate regulation in diabetic patients and ultimately improve patient outcomes.

This study provides valuable scientific data on the potential metabolic effects of Picralima nitida, especially its effect on serum glycated hemoglobin and phosphate levels. Having examined these biomarkers in animal models, the study can open the way to new clinical studies of the plant's role in treating diabetes.

The results can be beneficial for researchers, medical workers, and politicians, supporting the development of therapeutic interventions under plant conditions. Furthermore, if Picralima nitida demonstrates positive effects on glycemic control and phosphate metabolism, it could be a cost-effective alternative for people living in regions where access to conventional diabetes medications is limited.

Furthermore, this study could contribute to a better understanding of how phytochemicals interact with metabolic pathways, which could lead to the identification of novel bioactive compounds with anti-diabetic properties.

CHAPTER TWO

This chapter provides a comprehensive review of existing literature relevant to the study. It explores the botanical characteristics and pharmacological properties of *Picralima nitida*, the physiology of glycated hemoglobin (HbA1c) and serum phosphate concentrations, and previous research on the impact of medicinal plants on these biomarkers in male Wistar rats. The chapter also examines the methodologies used in related studies and identifies gaps in the literature that justify the current research.

2.1 *Picralima nitida*: Botanical and Pharmacological Profile

2.1.1 Botanical Description

Picralima nitida,which is also known as the "bitter bean" or "African peach," belongs to the Apocynaceae family. It is a small to medium-sized tree that is found in the West and Central Africa, flourishing in tropical and subtropical regions. The tree produces green, oblong fruits that turn yellow upon ripening, it contains numerous seeds, which are the primary source of its bioactive compounds. Various parts of the plant, which includes the seeds, bark, and leaves, have been extensively utilized in African traditional medicine for their therapeutic properties. Traditional medicine applications of *Picralima nitida* include the treatment of fever, malaria, gastrointestinal disorders, hypertension, and pain management (GH Schmelzer,2018). The medicinal value of *Picralima nitida* is primarily attributed to its rich bioactive compound composition. Bioactive compound analyses have identified a wide range of bioactive compounds, including alkaloids, flavonoids, tannins, saponins, glycosides, terpenoids, and steroids. Among these, the indole alkaloids—such as akuammine, akuammidine, akuammicine, and picraline—are particularly significant for their pharmacological activities (Mu Anyanwu, Okoye et al., 2017). These alkaloids have been found to interact with opioid receptors, contributing to the plant's potent analgesic and anti-inflammatory effects. Additionally, flavonoids and tannins present in *Picralima nitida* exhibit strong antioxidant properties, which may play a crucial role in protecting against oxidative stress and metabolic disorders (Fadare et al., 2021).

Figure2.1: Picralima nitida

Traditionally, various parts of the plant have been used to manage a wide range of ailments. The seeds are commonly used in the treatment of fever, malaria, and gastrointestinal disorders, while the bark and leaves are used for managing hypertension, dysentery, and inflammatory conditions. Herbal extracts of the seeds and bark are often prepared as herbal remedies for pain relief and infectious diseases. Due to its antimicrobial properties, *Picralima nitida* is also applied in the treatment of bacterial and fungal infections (A Alongnon, Spoil, et al., 2023). Furthermore, recent studies suggest that the plant possesses potential hypoglycemic and lipid-modulating effects, making it a Prospect for further research in metabolic disease management, including diabetes and cardiovascular disorders (Oyedemi et al., 2019).

The plant's diverse bioactive compounds suggest possible mechanisms by which it may influence blood glucose levels, including modulation of insulin secretion, enhancement of glucose uptake, and reduction of oxidative stress (Nathan et al., 2007). However, existing research primarily focuses on its traditional applications, with few experimental studies evaluating its direct impact on biochemical markers related to diabetes and phosphate metabolism. This knowledge gap underscores the need for further experimental and clinical investigations to establish the efficacy, safety, and pharmacokinetics of *Picralima nitida* in metabolic disease management.

2.1.2 **Pharmacological Effects**

Extracts of *Picralima nitida* have been shown to possess diverse pharmacological properties, such as blood sugar-lowering, anti-inflammatory, antioxidant, antimicrobial, and neuroprotective effects . These biological effects are primarily attributed to the plant's rich bioactive compound composition, particularly its alkaloids, flavonoids, and terpenoids, which have demonstrated potential therapeutic relevance in various disease models.

Hypoglycemic and Metabolic Effects

The hypoglycemic potential of *Picralima nitida* is believed to be Influenced through its alkaloid content, which has been linked to the modulation of key metabolic processes, including glucose regulation and lipid metabolism (Fadare et al., 2021). Several studies suggest that the bioactive compounds present in the plant can influence insulin sensitivity, enhance glucose uptake, and regulate hepatic glucose production. These mechanisms are critical for maintaining Normal blood sugar and preventing complications associated with metabolic disorders such as diabetes mellitus. Alkaloids like akuammidine and akuammicine may play a role in pancreatic β-cell function, promoting insulin secretion and improving glucose homeostasis (Mbiantcha et al., 2018).

In addition, flavonoids and tannins in *Picralima nitida* exhibit strong antioxidant properties, which could help mitigate oxidative stress—a major contributor to insulin resistance and β-cell dysfunction (Oyedemi et al., 2019). The antioxidant effects of these bioactive compound may reduce the formation of advanced glycation end-products (AGEs), which are associated with increased glycated hemoglobin (HbA1c) levels and diabetic complications. However, while the plant has demonstrated hypoglycemic potential in preliminary studies, its specific effects on

HbA1c, a key marker of long-term glucose control, remain largely unexplored and warrant further investigation.

2.1.3 Anti-Inflammatory and Antioxidant Properties

Chronic inflammation and oxidative stress are major contributors to metabolic and neurodegenerative disorders. Several studies have confirmed the anti-inflammatory effects of *Picralima nitida*, largely attributed to its alkaloids and flavonoids, which can modulate inflammatory pathways by inhibiting pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Okoye et al., 2014). These anti-inflammatory properties suggest potential applications in managing conditions such as arthritis, cardiovascular disease, and metabolic syndrome.

The plant's strong antioxidant activity, primarily due to its flavonoids and polyphenols, has been shown to remove free radicals and protect cells from oxidative damage (Egharevba & Kunle, 2010). This antioxidant potential is particularly relevant in metabolic disorders, where oxidative stress can accelerate complications such as nephropathy, retinopathy, and neuropathy in diabetic patients.

2.1.4 Antimicrobial and Neuroprotective Effects

The antimicrobial potential of *Picralima nitida* has been studied, with research demonstrating its effect against bacterial and fungal infections (Gbedema et al., 2006). The alkaloid-rich extracts have shown inhibitory effects against common pathogens, including *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*, suggesting its potential as a natural antimicrobial agent (Akinpelu et al., 2015).

Additionally, *Picralima nitida* has been reported to exhibit neuroprotective properties, with its bioactive compounds interacting with opioid receptors to produce analgesic effects. These findings suggest a potential role in managing neurological disorders, pain, and neurodegenerative diseases such as Alzheimer's and Parkinson's (Mbiantcha et al., 2018).

2.2 Historical Perspective on Glycated Hemoglobin and Serum Phosphate

2.2.1 Glycated Hemoglobin (HbA1c)

The history of glycated hemoglobin (HbA1c) dates back to the mid-20th century, when researchers began studying abnormal hemoglobin structures and their relationship to metabolic diseases. In 1958, Huisman and Meyering first identified different hemoglobin fractions through

electrophoretic separation, but the significance of these fractions remained unclear at the time. It was not until 1968 that Rahbar et al. made a groundbreaking discovery—an elevated level of one such fraction, later identified as HbA1c, in the blood of diabetic patients. This finding marked the beginning of its significance as a potential biomarker for diabetes management.

Further research throughout the 1970s established that HbA1c results from the non-enzymatic glycation of hemoglobin, a process where glucose binds to hemoglobin in red blood cells. Studies confirmed a direct correlation between HbA1c levels and blood glucose concentrations over time, reinforcing its potential as a marker for long-term glycemic control. By the late 1970s, researchers had developed more precise techniques, such as high-performance liquid chromatography (HPLC), to measure HbA1c accurately, paving the way for its clinical adoption.

The 1980s and 1990s were pivotal decades in the clinical validation of HbA1c. The landmark Diabetes Control and Complications Trial (DCCT) (1993) and the United Kingdom Prospective Diabetes Study (UKPDS) (1998) provided compelling evidence that maintaining lower HbA1c levels significantly reduced the risk of diabetes-related complications, including retinopathy, nephropathy, and cardiovascular diseases. These findings cemented the role of HbA1c as an essential tool in diabetes management, influencing treatment protocols worldwide.

In 2010, the American Diabetes Association (ADA) officially endorsed HbA1c as a diagnostic criterion for diabetes, setting a threshold of ≥6.5% for diabetes diagnosis. This decision was based on extensive research demonstrating the strong predictive value of HbA1c in identifying individuals at risk of developing diabetes and its complications. Since then there has been advancements in analytical methodologies, such as immunoassays and capillary electrophoresis, have further improved the accuracy and reliability of HbA1c measurement, making it the piller of diabetes diagnosis and monitoring.

Despite its widespread use, the interpretation of HbA1c levels remains an area of ongoing research. Factors such as genetic variations, ethnic differences, and conditions affecting red blood cell lifespan (e.g., anemia, hemoglobinopathies) can influence HbA1c readings, necessitating additional biomarkers in certain clinical scenarios. Researchers continue to explore alternative markers, such as glycated albumin and continuous glucose monitoring (CGM), to complement HbA1c and improve personalized diabetes care.

2.2.2 Serum Phosphate

The historical understanding of phosphate metabolism has evolved significantly over the centuries, reflecting more advancements in physiology, endocrinology, and nephrology. Early medical observations recognized phosphate as a vital component of bone health and cellular function, but its precise regulatory mechanisms remained poorly understood.

In the early 20th century, research into renal function highlighted the kidney's important role in maintaining phosphate homeostasis. Investigators discovered that phosphate levels were influenced by diet, renal excretion, and bone turnover, but the hormonal regulators of phosphate balance remained unknown. The discovery of parathyroid hormone (PTH) in the 1920s was a major breakthrough, as researchers found that PTH increased renal phosphate excretion while promoting calcium retention. This finding provided early insights into the intricate relationship between calcium and phosphate metabolism.

By the mid-20th century, vitamin D's role in phosphate regulation became clearer. Studies demonstrated that vitamin D enhances intestinal phosphate absorption, further integrating phosphate metabolism with calcium homeostasis and bone health. The growing recognition of phosphate's systemic effects led to further investigations into its role beyond skeletal integrity, including its impact on cellular energy production (via ATP), enzyme activity, and intracellular signaling.

A major advancement in phosphate research occurred in the early 2000s with the identification of fibroblast growth factor 23 (FGF23), a hormone primarily secreted by osteocytes. FGF23 was found to regulate renal phosphate excretion and vitamin D metabolism, introducing a novel regulatory pathway that linked phosphate homeostasis to bone health, cardiovascular function, and metabolic disorders. FGF23's discovery significantly expanded the understanding of phosphate regulation and its implications in diseases such as chronic kidney disease (CKD), where phosphate retention contributes to cardiovascular morbidity and mortality.

In recent years, serum phosphate levels have gained increasing attention in metabolic and endocrine research. Studies have shown that phosphate dysregulation is not only a concern in kidney disease but also plays a role in conditions such as diabetes, cardiovascular disease, and osteoporosis. increased serum phosphate levels, often associated with CKD and hyperphosphatemia, have been linked to vascular calcification and increased cardiovascular risk. Conversely, hypophosphatemia, resulting from malnutrition, genetic disorders, or excessive

phosphate excretion, can lead to muscle weakness, bone abnormalities, and impaired cellular function.

As research continues, clinicians and scientists are exploring new therapeutic strategies to manage phosphate imbalances. Phosphate binders, dietary modifications, and targeted therapies such as FGF23 inhibitors are being investigated for their potential to mitigate phosphate-related complications in various diseases. Furthermore, advancements in phosphate measurement techniques have improved diagnostic accuracy, allowing for more precise monitoring of phosphate levels in clinical practice.

The historical progression of glycated hemoglobin and serum phosphate research highlights the dynamic nature of biomedical science. HbA1c has evolved from an incidental discovery to a globally recognized biomarker for diabetes diagnosis and management, shaping modern endocrinology and metabolic medicine. Similarly, the understanding of phosphate metabolism has advanced from basic physiological observations to a complex regulatory network involving hormones such as PTH, vitamin D, and FGF23. Ongoing research in both fields continues to refine clinical applications, improve diagnostic accuracy, and expand therapeutic strategies, ultimately enhancing patient care and disease management.

2.3 Glycated Hemoglobin (HbA1c)

2.3.1 Physiological Role and Clinical Significance

Glycated hemoglobin (HbA1c) is a biomarker formed through the non-enzymatic glycation of hemoglobin in circulating red blood cells. This process occurs when glucose molecules in the bloodstream bind irreversibly to hemoglobin, resulting in the formation of HbA1c. Since red blood cells have an average lifespan of approximately 120 days, (Nathan et al., 2007) Unlike fasting blood glucose or postprandial glucose tests, which provide a snapshot of blood sugar levels at a specific moment, HbA1c gives an integrated view of glucose regulation over time. HbA1c levels provide a cumulative measure of blood glucose concentrations over the preceding two to three months.

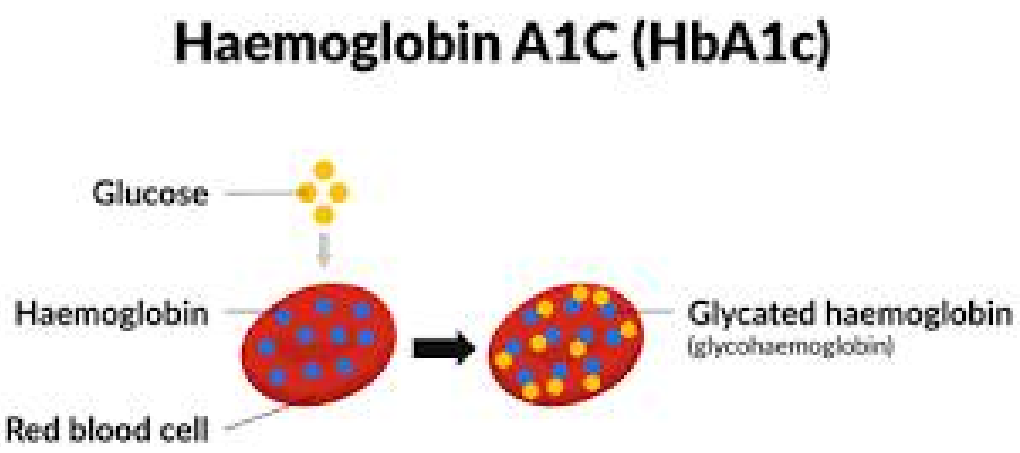

Figure2.2: Glycated Hemoglobin

HbA1c is a critical parameter in the diagnosis and management of diabetes mellitus, with higher levels indicating chronic hyperglycemia and an increased risk of diabetes-related complications. The American Diabetes Association (ADA) classifies an HbA1c level of 5.7–6.4% as indicative of prediabetes, while levels ≥6.5% are diagnostic of diabetes (ADA, 2020). Persistent elevation of HbA1c is associated with an increased risk of microvascular and macrovascular complications, including diabetic neuropathy, nephropathy, retinopathy, and cardiovascular diseases (Forbes & Cooper, 2013).

Beyond diabetes, HbA1c levels have been explored as a predictor of other metabolic and inflammatory conditions. Studies suggest that elevated HbA1c may be linked to oxidative stress, inflammatory responses, and lipid abnormalities, which further contribute to the pathogenesis of atherosclerosis and other chronic disorders (Singh et al., 2020). Given its significance, interventions that lower HbA1c levels, including lifestyle modifications and pharmacological treatments, are widely recommended to prevent disease progression and improve patient outcomes.

2.3.2 Importance of HbA1c in Diabetes Management

For individuals with diabetes, maintaining optimal HbA1c levels is essential to prevent complications. The target HbA1c level for most diabetics is:

I. <7.0% (53 mmol/mol) for general diabetes management.
II. <6.5% (48 mmol/mol) for younger patients with a lower risk of hypoglycemia.
III. <8.0% (64 mmol/mol) for elderly patients or those with comorbidities.

Regular monitoring of HbA1c allows healthcare providers to adjust treatment plans, including medication, diet, and lifestyle modifications.

2.3.3 Limitations of HbA1c Testing

Despite its utility, HbA1c testing has some limitations:

I. It may be unreliable in conditions affecting red blood cell turnover (e.g., hemolytic anemia, chronic kidney disease).

II. It does not detect acute fluctuations in blood glucose, necessitating complementary tests like fasting blood glucose or continuous glucose monitoring in some cases.

2.3.4 Factors Affecting HbA1c Levels

Several physiological and pathological factors influence HbA1c levels beyond glycemic control. These include:

I. **Dietary Intake and Nutritional Status**: **Diet and nutrition:** High carbohydrate intake and frequent hyperglycemia increase HbA1c levels.

II. Low glycemic index diets, fiber-rich foods, and protein intake can improve glycemic control and lower HbA1c.

III. Micronutrient deficiencies (e.g., iron, vitamin B12) can impact RBC turnover and glycation processes. (Jenkins et al., 2002).

IV. **Insulin Sensitivity and Secretion**: Insulin resistance, as seen in type 2 diabetes and metabolic syndrome, leads to prolonged hyperglycemia, thereby increasing HbA1c levels (DeFronzo et al., 2009).

V. **Glucose Concentration and Metabolism:** HbA1c levels primarily depend on blood glucose concentration. Chronic hyperglycemia leads to increased glycation of hemoglobin, while improved glycemic control lowers HbA1c levels. Insulin sensitivity, pancreatic beta-cell function, and hepatic glucose production all play a role in determining blood glucose levels.

VI. **Red blood cell Lifespan and Turnover**: Conditions affecting red blood cell turnover, such as anemia, hemolysis, and chronic kidney disease, may alter HbA1c levels independently of glucose control (Cohen et al., 2008). Since HbA1c reflects glucose exposure over the life cycle of red blood cells (typically 120 days), alterations in RBC lifespan significantly impact HbA1c values. Conditions that shorten RBC

lifespan (e.g., hemolytic anemia) lower HbA1c levels, while conditions that prolong RBC survival (e.g., iron deficiency anemia) may falsely elevate HbA1c.

VII. **Oxidative Stress and Inflammation**: Chronic inflammation and oxidative stress can exacerbate glucose dysregulation, contributing to elevated HbA1c levels in individuals with metabolic disorders (Brownlee, 2001).

VIII. **Anemia:** Iron deficiency anemia, vitamin B12 deficiency, and folate deficiency can prolong RBC lifespan, leading to increased HbA1c levels, while hemolytic anemia and chronic blood loss decrease HbA1c values.

IX. **Hemoglobinopathies:** Variants such as sickle cell disease and thalassemia can interfere with HbA1c measurement and affect glycation rates.

X. **Renal Dysfunction:** Chronic kidney disease (CKD) alters HbA1c levels due to anemia, reduced erythropoiesis, and altered glycation rates. Additionally, uremia can interfere with HbA1c assays, making alternative biomarkers like glycated albumin more reliable in advanced CKD.

XI. **Physical activities:** Regular exercise enhances insulin sensitivity and glucose uptake, leading to improved glycemic control and lower HbA1c values. Sedentary lifestyles contribute to insulin resistance and elevated HbA1c levels.

XII. **Alcohol:** Chronic alcohol consumption may cause hypoglycemia, while excessive intake can contribute to insulin resistance.

XIII. **Smoking:** Nicotine increases insulin resistance, leading to higher HbA1c levels in smokers compared to non-smokers.

Given the increasing interest in plant-based therapies for diabetes management, medicinal plants with hypoglycemic properties have been studied for their potential to reduce HbA1c levels. These plants may exert their effects through various mechanisms, including:

I. **Enhancing Insulin Secretion**: Certain phytochemicals stimulate pancreatic β-cell function, increasing insulin production (Patel et al., 2012).

II. **Improving Glucose Uptake**: Some bioactive compounds promote glucose uptake in peripheral tissues by upregulating glucose transporter proteins (GLUTs) (Hawley et al., 2010).

III. **Modulating Oxidative Stress**: Antioxidant-rich plants help counteract oxidative damage, thereby reducing insulin resistance and improving glucose metabolism (Sabu & Kuttan, 2002).

IV. **Inhibiting Carbohydrate Digestion and Absorption**: Some plant extracts slow down carbohydrate breakdown and glucose absorption in the intestine, leading to lower postprandial glucose spikes (Tundis et al., 2010)

2.4 Role of Phosphate in Metabolism

Phosphate is a vital inorganic mineral that plays a fundamental role in numerous physiological processes, including energy metabolism, skeletal development, and acid-base homeostasis. It is an essential component of adenosine triphosphate (ATP), which serves as the primary energy currency of the cell, as well as nucleotides, phospholipids, and secondary messengers involved in signal transduction (Bergwitz & Jüppner, 2010).

Serum phosphate levels are tightly regulated by a complex interplay of hormonal and renal mechanisms to maintain homeostasis. Key regulators include:

I. **Parathyroid Hormone (PTH)**: PTH increases renal phosphate excretion while promoting calcium reabsorption, thereby maintaining an appropriate calcium-to-phosphate balance (Shimada et al., 2004).

II. **Fibroblast Growth Factor 23 (FGF23)**: Secreted primarily by osteocytes, FGF23 enhances renal phosphate excretion while suppressing vitamin D activation, thus reducing intestinal phosphate absorption (Martin et al., 2012).

III. **Vitamin D (Calcitriol)**: Active vitamin D enhances intestinal phosphate absorption and facilitates bone mineralization by promoting the deposition of calcium and phosphate into the skeletal matrix (Girgis et al., 2013).

Given its importance in metabolic regulation, disruptions in phosphate homeostasis can have significant health implications. Hypophosphatemia (low serum phosphate levels) can lead to muscle weakness, impaired energy metabolism, and bone demineralization, whereas hyperphosphatemia (elevated serum phosphate levels) has been linked to vascular calcification, chronic kidney disease (CKD), and cardiovascular dysfunction (Calvo et al., 2013).

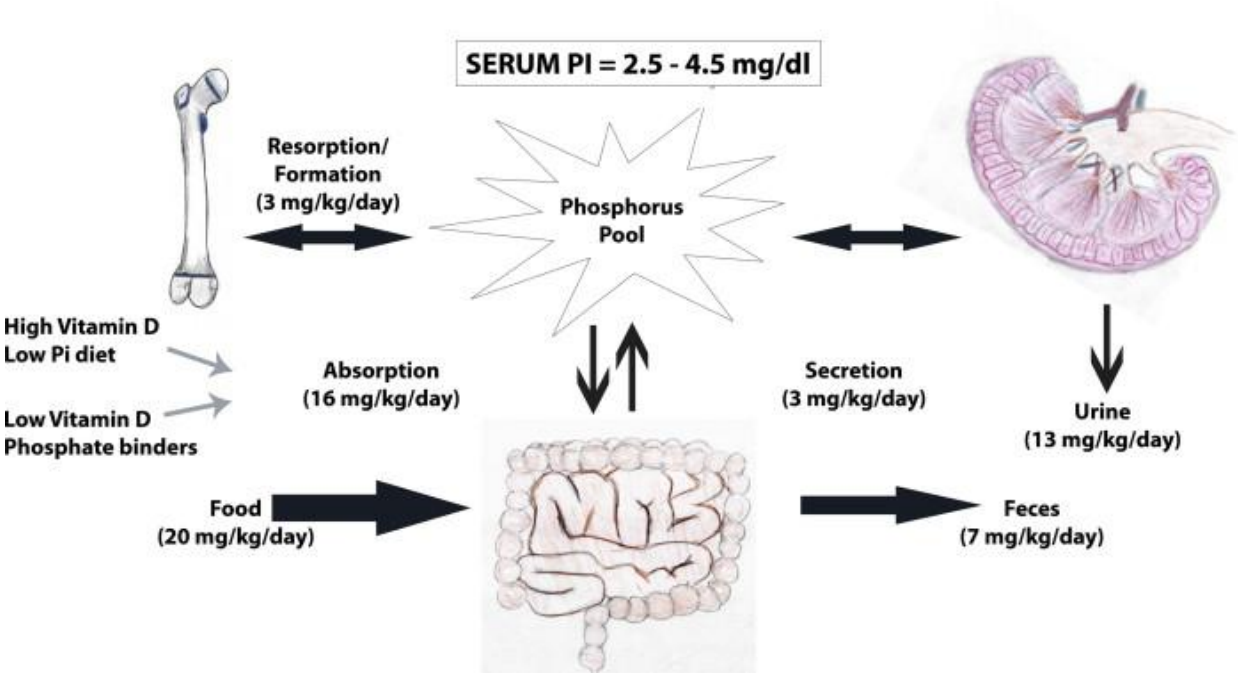

Figure2.3: Overview of Phosphate Metabolism

2.4.1 Factors Affecting Serum Phosphate Levels

Serum phosphate levels are regulated by a complex interplay of dietary intake, hormonal control, renal function, bone metabolism, and various physiological conditions (Berndt & Kumar, 2009). Maintaining phosphate homeostasis is critical for numerous biological processes, including energy metabolism, bone mineralization, and cellular signaling. Several factors can influence serum phosphate concentrations, leading to either hyperphosphatemia (elevated phosphate levels) or hypophosphatemia (low phosphate levels), both of which have significant clinical implications (Shah et al., 2015).

A. Renal Function and Phosphate Excretion

The kidneys play a pivotal role in phosphate balance by regulating its reabsorption and excretion. Approximately 85% of the phosphate filtered by the glomeruli is reabsorbed in the proximal tubule through sodium-phosphate co-transporters (NaPi-IIa and NaPi-IIc) (Moe, 2008). The efficiency of this process is influenced by:

I. **Chronic Kidney Disease (CKD):** Reduced renal function impairs phosphate excretion, leading to hyperphosphatemia. This condition is commonly associated with secondary hyperparathyroidism, vascular calcification, and increased cardiovascular risk (Isakova et al., 2011).

II. **Parathyroid Hormone (PTH):** PTH plays a critical role in phosphate regulation by decreasing renal phosphate reabsorption, thereby increasing phosphate excretion (phosphaturia) (Fukagawa et al., 2017). Elevated PTH levels, often seen in chronic kidney disease and hyperparathyroidism, contribute to phosphate loss and hypophosphatemia.

III. **Fibroblast Growth Factor 23 (FGF23):** Secreted by osteocytes, FGF23 enhances renal phosphate excretion while inhibiting active vitamin D production, which reduces intestinal phosphate absorption (Shimada et al., 2004). Elevated FGF23 levels are frequently observed in CKD and disorders affecting phosphate metabolism.

B. Dietary Intake and Gastrointestinal Absorption

Phosphate homeostasis is heavily influenced by dietary intake and its subsequent absorption in the gastrointestinal tract (Calvo et al., 2013). Several factors affect phosphate absorption:

I. **Dietary Phosphate Content:** Foods rich in phosphate, such as dairy products, meats, legumes, nuts, and processed foods containing phosphate additives, can elevate serum phosphate levels (Ritz et al., 2012). Conversely, a phosphate-deficient diet may lead to hypophosphatemia.

II. **Vitamin D Status:** Active vitamin D (1,25-dihydroxyvitamin D) enhances phosphate absorption by upregulating phosphate transporters in the intestine (Kemi et al., 2006). Vitamin D deficiency reduces phosphate absorption, contributing to hypophosphatemia.

III. **Gastrointestinal Disorders:** Conditions such as celiac disease, Crohn's disease, and chronic diarrhea can impair phosphate absorption, leading to low serum phosphate levels (de Menezes Montenegro et al., 2020).

IV. **Phosphate Binders:** Commonly used in patients with CKD, phosphate binders (e.g., calcium acetate, sevelamer) reduce phosphate absorption in the intestine, helping control hyperphosphatemia (Qunibi, 2010).

C. Bone Metabolism and Skeletal Phosphate Storage

Bone acts as a major reservoir for phosphate, and its metabolism significantly influences serum phosphate levels (Razzaque, 2009). Factors affecting bone phosphate turnover include:

I. **Osteoclastic and Osteoblastic Activity:** Bone remodeling involves continuous deposition and resorption of phosphate-containing hydroxyapatite. Increased bone resorption (e.g., in osteoporosis or metastatic bone disease) can elevate serum phosphate levels, while impaired bone mineralization (e.g., in rickets or osteomalacia) may lead to hypophosphatemia (Sharma et al., 2013).

II. **Calcium-Phosphate Balance:** Serum phosphate levels are closely linked to calcium regulation via the actions of PTH, vitamin D, and FGF23. Disruptions in calcium-phosphate homeostasis can lead to metabolic bone disorders and abnormalities in phosphate levels (Kuro-o, 2010).

D. Hormonal Regulation

Various hormones influence phosphate homeostasis by modulating its absorption, excretion, and bone storage. Key regulatory hormones include:

I. **Parathyroid Hormone (PTH):** Increases renal phosphate excretion and enhances bone resorption, thereby elevating serum calcium while lowering phosphate levels (Silver et al., 2012).

II. **Fibroblast Growth Factor 23 (FGF23):** Promotes phosphate excretion and inhibits vitamin D activation, thereby reducing both serum phosphate and intestinal absorption (Shimada et al., 2004).

III. **Calcitonin:** Secreted by the thyroid gland, calcitonin inhibits bone resorption and enhances renal phosphate excretion, lowering serum phosphate levels (Fukumoto & Yamashita, 2002).

IV. **Growth Hormone and Insulin-like Growth Factor-1 (IGF-1):** Promote bone growth and phosphate retention, leading to increased serum phosphate levels (Baxter, 2014).

E. Medications and Pharmacological Effects

Certain medications can significantly impact serum phosphate levels (Liu et al., 2020):

I. **Diuretics:** Loop diuretics (e.g., furosemide) promote phosphate excretion, leading to hypophosphatemia, whereas thiazide diuretics may cause hyperphosphatemia (Bello et al., 2018).

II. **Bisphosphonates:** Used to treat osteoporosis, bisphosphonates can lower serum phosphate levels by reducing bone resorption (Papapoulos, 2008).
III. **Antacids and Proton Pump Inhibitors (PPIs):** Chronic use of aluminum- or magnesium-containing antacids can bind dietary phosphate, reducing its absorption and leading to hypophosphatemia (Perwad & Portale, 2011).
IV. **Chemotherapy and Immunosuppressive Drugs:** Certain cancer therapies and immunosuppressants can induce tumor lysis syndrome, causing hyperphosphatemia due to rapid cell breakdown (Howard et al., 2011).

F. Physiological and Pathological Conditions

Several physiological states and medical conditions influence serum phosphate levels (Kestenbaum et al., 2010):

I. **Aging:** Older adults often exhibit reduced renal phosphate excretion and altered hormonal regulation, which can contribute to higher phosphate levels.
II. **Diabetes Mellitus:** Poor glycemic control in diabetes can lead to alterations in phosphate balance due to polyuria and osmotic diuresis, potentially causing hypophosphatemia (Blaine et al., 2015).
III. **Liver Disease:** Since the liver is involved in vitamin D metabolism, chronic liver disease can lead to impaired phosphate absorption and hypophosphatemia (Sheikh et al., 2018).
IV. **Acid-Base Imbalance:** Metabolic acidosis enhances phosphate excretion, while metabolic alkalosis promotes phosphate retention (Giachelli, 2009).

G. Acute and Chronic Illnesses

I. **Sepsis and Critical Illness:** Severe infections and systemic inflammation can lead to shifts in phosphate distribution, causing hypophosphatemia due to increased cellular uptake (Arai et al., 2013).
II. **Tumor Lysis Syndrome:** A condition where rapid destruction of tumor cells releases large amounts of phosphate into circulation, leading to severe hyperphosphatemia (Cairo & Bishop, 2004).

Serum phosphate levels are tightly regulated by the kidneys, gastrointestinal absorption, bone metabolism, hormonal control, and external factors such as diet and medications. Disruptions in

phosphate homeostasis can lead to metabolic disorders with significant clinical consequences. Understanding these factors is essential for diagnosing and managing conditions related to phosphate imbalances, ensuring optimal physiological function and overall health (Shah et al., 2015).

2.5 Previous Studies on Medicinal Plants and Metabolic Biomarkers

Medicinal plants have long been explored for their potential to regulate metabolic biomarkers, particularly in the management of diabetes, mineral metabolism, and cardiovascular health. A growing body of research highlights the ability of various plant species to modulate glycated hemoglobin (HbA1c) and serum phosphate levels through multiple biochemical and physiological mechanisms, such as enhancing insulin sensitivity, promoting renal phosphate excretion, reducing oxidative stress, and influencing hormonal regulation. These effects are often attributed to the presence of bioactive compounds, including polyphenols, flavonoids, alkaloids, terpenoids, and other phytochemicals that interact with metabolic pathways.

Several medicinal plants have been extensively studied for their potential role in improving glucose metabolism and phosphate homeostasis:

I. Moringa oleifera: Renowned for its broad-spectrum medicinal properties, Moringa oleifera has been widely recognized for its antidiabetic and mineral-regulating effects. Studies have reported its ability to lower blood glucose levels and improve calcium-phosphate metabolism due to its rich composition of polyphenols, flavonoids, glucosinolates, and essential minerals (Stohs & Hartman, 2015). The plant has been found to enhance insulin sensitivity, reduce postprandial glucose spikes, and mitigate oxidative stress, which collectively contribute to better glycemic control and phosphate balance.

II. Vernonia amygdalina: Commonly known as bitter leaf, Vernonia amygdalina has demonstrated significant hypoglycemic effects by enhancing insulin secretion and promoting glucose uptake in peripheral tissues (Atangwho et al., 2013). Its potential role in phosphate metabolism has also been suggested, as it may influence renal function by enhancing phosphate excretion and modulating electrolyte balance. Additionally, its antioxidant and anti-inflammatory properties contribute to improved metabolic stability, making it a promising candidate for further research in diabetes and mineral homeostasis.

III. Garcinia kola: Garcinia kola has been traditionally used in African herbal medicine for various ailments, including diabetes management. Studies have shown that it exerts both hypoglycemic and nephroprotective effects, suggesting its potential role in phosphate homeostasis by preventing renal dysfunction and supporting overall metabolic balance (Adaramoye et al., 2005). The plant's bioactive constituents, such as flavonoids and biflavonoids, exhibit antioxidative and anti-inflammatory properties that may protect pancreatic beta cells, enhance insulin secretion, and regulate mineral metabolism.

IV. Momordica charantia: Commonly referred to as bitter melon, Momordica charantia has been widely studied for its antidiabetic properties. It contains bioactive compounds such as charantin, vicine, and polypeptide-p, which have been shown to lower blood glucose by stimulating insulin secretion, enhancing glucose uptake, and inhibiting intestinal glucose absorption (Patel et al., 2012). Additionally, its mineral-modulating properties have been linked to its influence on renal function and electrolyte balance, making it a promising candidate for further investigation in phosphate metabolism and metabolic disorders.

2.6 Research Gaps and Rationale

2.6.1 Research Gaps

The study of medicinal plants as potential therapeutic agents for metabolic disorders has gained increasing attention. However, despite the ethnomedicinal significance of *Picralima nitida*, there remains a substantial lack of research regarding its influence on glycated hemoglobin (HbA1c) and serum phosphate concentrations. The following key research gaps highlight the need for further investigation:

I. **Limited Empirical Evidence on the Effects of *Picralima nitida* on HbA1c**
Glycated hemoglobin (HbA1c) is an essential biomarker for long-term glycemic control and is widely used in the diagnosis and management of diabetes mellitus (American Diabetes Association, 2021). While *Picralima nitida* has been traditionally used for managing diabetes in African herbal medicine, most available studies have focused on its hypoglycemic properties rather than its long-term impact on HbA1c. There is a clear need for controlled experimental studies to determine whether *Picralima nitida* can

significantly reduce HbA1c levels over extended periods, thereby offering sustained glycemic benefits beyond immediate blood glucose reduction (Okoye et al., 2014).

II. **Unexplored Relationship Between *Picralima nitida* and Serum Phosphate Regulation**

Serum phosphate plays a crucial role in metabolic processes, including energy transfer, bone mineralization, and cell signaling (Razzaque, 2020). Dysregulation of phosphate homeostasis is often observed in conditions such as chronic kidney disease (CKD), diabetes, and cardiovascular disorders (Vervloet et al., 2017). However, no existing studies have examined the influence of *Picralima nitida* on serum phosphate levels. Given the interconnected nature of glucose metabolism and mineral homeostasis, it is imperative to assess whether this plant exerts any significant effects on phosphate regulation.

III. **Lack of Mechanistic Insights and Pharmacological Pathways**

The bioactive compounds in *Picralima nitida*, such as alkaloids, have been investigated for their antimalarial, analgesic, and anti-inflammatory properties (Adotey et al., 2011). However, the precise pharmacological mechanisms through which these compounds influence glucose metabolism and phosphate homeostasis remain largely unexplored. Does *Picralima nitida* enhance insulin secretion, improve insulin sensitivity, or act through other metabolic pathways? Additionally, its potential effects on renal phosphate excretion or hormonal regulation of phosphate balance (e.g., through fibroblast growth factor 23 [FGF23] or parathyroid hormone [PTH]) remain unknown. Addressing these mechanistic gaps will provide a clearer understanding of how *Picralima nitida* exerts its potential effects.

IV. **Scarcity of Well-Controlled In Vivo Studies**

Existing research on *Picralima nitida* is primarily limited to in vitro studies and anecdotal ethnobotanical reports. While a few in vivo studies have assessed its toxicity and general pharmacological effects (Adaramoye et al., 2005), there is a distinct lack of structured preclinical trials using animal models to evaluate its efficacy in metabolic regulation. Conducting a well-designed study in Wistar rats, a widely accepted animal model for metabolic research, will provide valuable insights into its biological effects, toxicity profile, and therapeutic potential.

V. **Comparative Analysis with Conventional Therapeutic Agents**

The efficacy of *Picralima nitida* in lowering HbA1c and modulating serum phosphate has not been compared with conventional antidiabetic drugs (e.g., metformin, insulin) or phosphate-regulating agents (e.g., phosphate binders, calcitriol). Understanding how its effectiveness compares with standard treatments will determine whether it can be used as a complementary or alternative therapy for managing metabolic disorders. A comparative study will also help elucidate whether *Picralima nitida* offers unique advantages, such as fewer side effects or broader metabolic benefits.

2.6.2 Rationale for the Study

Given these research gaps, this study aims to systematically analyze the effects of *Picralima nitida* on glycated hemoglobin and serum phosphate concentrations in male Wistar rats. The rationale for this study is driven by several key factors:

I. **Bridging the Knowledge Gap in traditional medicine and Modern Pharmacology**

Picralima nitida has been traditionally used for treating diabetes and other ailments in African medicine (Okoye et al., 2014). However, modern pharmacological research has yet to validate its efficacy in regulating HbA1c and phosphate homeostasis. By integrating ethnobotanical knowledge with scientific experimentation, this study will contribute to the validation and potential mainstream adoption of this medicinal plant.

II. **Potential for Development of Novel Plant-Based Therapeutics**

With increasing interest in plant-derived bioactive compounds for managing chronic diseases, there is a need to identify effective herbal interventions that can complement existing pharmacological treatments (Atangwho et al., 2013). If *Picralima nitida* is found to exert beneficial effects on HbA1c and phosphate metabolism, it could serve as a foundation for the development of plant-based therapeutic formulations, reducing reliance on synthetic drugs.

III. **Addressing the Burden of Diabetes and Associated Complications**

Diabetes mellitus and its complications, including mineral imbalances and kidney dysfunction, remain major global health concerns (International Diabetes Federation, 2021). Given the role of phosphate in metabolic and cardiovascular health (Vervloet et al.,

2017), understanding how *Picralima nitida* affects both HbA1c and phosphate levels will provide a broader perspective on its potential in holistic metabolic management.

IV. **Expanding Preclinical Evidence to Support Future Clinical Trials**

Preclinical studies in animal models are essential for establishing the safety and efficacy of new therapeutic agents before progressing to human trials. By using Wistar rats as an experimental model, this study will generate foundational data that could support subsequent clinical investigations into the role of *Picralima nitida* in metabolic regulation. If results are promising, this could pave the way for further pharmacokinetic and clinical studies in human populations.

V. **Integration of Traditional and Scientific Knowledge for Evidence-Based Medicine**

The study aligns with the growing movement toward integrating traditional medicine with modern biomedical research. Many plant-based therapies remain underutilized due to the lack of scientific validation. This research seeks to fill that gap by providing a structured, evidence-based approach to evaluating *Picralima nitida*, contributing to a more comprehensive understanding of its metabolic effects.

VI. **Exploring Synergistic or Novel Mechanisms of Action**

If *Picralima nitida* exhibits significant effects on HbA1c and serum phosphate, it will be crucial to explore whether its mechanisms of action differ from or complement those of existing antidiabetic and phosphate-regulating drugs. Identifying unique bioactive compounds and pathways could open new avenues for metabolic research and drug discovery.

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 EQUIPMENT AND APPARATUS:

I. Hand Gloves
II. Cotton wool
III. Chloroform
IV. Dissecting set
V. Methylated spirit
VI. Laboratory coat
VII. Sample container (Lithium heparin)
VIII. Refrigerator (Hisense Refrigerator, Model; REF302DR)
IX. Weighing scale (NEXT-SHINE,china, POC-P225-CA1)
X. Scissors
XI. Gavage
XII. Syringe (5ml and 10ml)
XIII. Lancets
XIV. Plain containers
XV. EDTA containers
XVI. Knife

3.2 MACHINES

The following machines were used for the study

I. Glucometer (ACCU-Check, United Kingdom)
II. Weighing scale, (NEXT-SHINE, China, Model: POC-P225-CA1)
III. Storage system (Haier Thermocool chest freezer (Model: HTF-319H))
IV. Grinder (SY-18B Industrial Dry Herbs Grinder)
V. Freeze Dryer (Biobase BK-FD10s Freeze Dryer (Xi'an, China))

3.3 ANIMAL EXPERIMENTAL STUDY

I. Forty (40) physiologically normal male albino Wistar rats without any morbid disorders were used as the subjects of this study. These rats were obtained from the Department of Anatomy, University of Benin, Nigeria. Upon arrival, the rats were acclimatized for one

week in a standard animal house under controlled environmental conditions, including a temperature of 22 ± 2°C, relative humidity of 50-60%, and a 12-hour light/dark cycle. The rats were housed in clean, well-ventilated plastic cages with soft bedding saw dusts, which was replaced regularly to maintain proper sanitation. They were fed with commercial chicken grower mash and had unrestricted access to clean water throughout the study period.

II. The rats were randomly divided into five groups based on their initial weight ranges: Group A (56.9g - 64.9g), Group B (66.4g - 72.7g), Group C (76.7g - 77.2g), Group D (77.2g - 80.76g), and Group E (82.9g - 90.93g). Each group consisted of eight rats, ensuring even distribution across the weight ranges. To facilitate easy identification, the rats were individually labeled using colored markers (GV) on specific body parts, including the hand, leg, back, head, and tail. Each rat was assigned a unique identification based on the part of the body where the color was applied.

III. During the experiment, the rats were closely monitored for any signs of distress, behavioral changes, or alterations in their physical condition. Their weights were recorded weekly to observe any significant changes that might indicate the effects of the experimental procedures. The rats were kept under optimal care, and all handling procedures were designed to minimize stress and maintain their well-being.

IV. At the end of the experimental period, the rats were sacrificed humanely using chloroform anesthesia, following ethical guidelines for animal research. Blood samples were collected via cardiac puncture where necessary, Samples were carefully stored in labeled lithium heparin containers and kept in a refrigerator to preserve their integrity until analysis.

This study was conducted following the ethical standards outlined by the relevant ethics board of the University of Benin. Throughout the study, efforts were made to ensure the humane treatment of the animals and to adhere strictly to ethical practices in biomedical research.

3.4 AQUEOUS EXTRACT PREPARATION

The *Picralima nitida* fruits used in this study were commercially obtained from the Ediaken (Uselu) market area of Benin City, Nigeria. A total of forty (40) fruits of varying sizes, each weighing between 0.2 - 0.5 kg, were purchased. The fruits were then transported to the Department of Plant Biology and Biotechnology, University of Benin, where they were identified and authenticated at the department's herbarium.

Following proper identification, the fruits were taken to the Department of Medical Biochemistry for further processing. The fruits were thoroughly washed with clean water to remove dirt and debris, ensuring that they were free of contaminants. Using sharp stainless-steel knives, the fruits were carefully peeled to remove the rinds. The white pulp was cut into smaller pieces while

ensuring that the seeds were completely removed to avoid any interference with the extraction process.

The cut pulp pieces were spread on clean trays and exposed to direct sunlight for **three (3) weeks** to achieve adequate drying. During this period, the pulp was regularly turned to ensure even drying and to prevent microbial growth. The drying process continued until the moisture content was visibly reduced, and the pulp had become significantly dry and brittle.

Once the drying process was complete, the dried pulp was mechanically ground into a fine powder using a SY-18B Industrial Dry Herbs Grinder. The grinding process was thorough to ensure that a smooth, fine powder was obtained, which would facilitate efficient extraction of bioactive compounds. The powdered pulp was then subjected to freeze-drying for an additional **one (1) week** to further reduce the moisture content and preserve the extract's integrity.

For the aqueous extraction, the powdered sample was soaked in distilled water at a ratio of 1:10 kg/L, following the method described by *De Campos et al.* (2020). The mixture was continuously stirred for **72 hours** to ensure maximum solubility of the bioactive components. After the soaking period, the slurry was filtered through several layers, including filter paper, cotton wool, and muslin cloth, to obtain a clear filtrate.

The clear filtrate was then freeze-dried using a Biobase BK-FD10s Freeze Dryer (Xi'an, China). This process involved sublimating the water content under low temperatures and pressure, resulting in a stable, dry extract. At the end of the freeze-drying process, a total yield of 637.6 g (12.6%) of the extract was obtained. The freeze-dried extract was stored in an airtight container at 40°C until further use to maintain its stability and prevent contamination.

Phytochemical analysis of the freeze-dried aqueous extract revealed the presence of significant bioactive compounds. The extract was found to contain 39.24 ± 0.45 mg GAE/g extract of total phenols and 24.39 ± 0.74 mg QE/g extract of flavonoids, as reported by *Ilenowa et al.* (2024). These bioactive components are known for their antioxidant properties and contribute to the therapeutic potential of the extract.

The preparation method ensured that the extract retained its bioactive properties while providing a safe and effective preparation for experimental use.

3.5 EXPERIMENTAL DESIGN

Forty (40) physiologically normal male albino Wistar rats, weighing between 56.9 g and 90.3 g, were used for this experimental study. The rats were obtained from the Department of Anatomy, University of Benin, Nigeria, and were acclimatized for three weeks. During the acclimatization period, the rats were housed in well-ventilated plastic cages, maintained under standard laboratory conditions with a 12-hour light/dark cycle, an ambient temperature of 25 ± 2°C, and

relative humidity of 50-60%. The cages were regularly cleaned, and sawdust bedding was changed periodically to maintain hygiene.

The rats were fed commercial rat feed (Chicken Grower Feed, Top Feeds Ltd, Nigeria) with an approximate nutrient composition of 16% crude protein, 7% crude fiber, 3% fat, 1% calcium, and 0.45% phosphorus. Clean drinking water was provided ad libitum. The initial fasting blood glucose levels of the rats were measured using a glucometer, with values ranging from 41 mg/dL to 136 mg/dL.

After acclimatization, the rats were grouped into five experimental groups based on their weights:

Group A: 56.9 g - 64.9 g
Group B: 66.4 g - 72.7 g
Group C: 76.7 g - 77.2 g
Group D: 77.2 g - 80.76 g
Group E: 82.9 g - 90.93 g

On the fourth week, the rats were marked using Gentian Violet (GV) for proper identification. Markings were made on specific body parts (head, hand, leg, tail, back, hand & back, head & back, and plain) to facilitate monitoring of individual weights, glucose levels, and extract dosage calculations.

3.6 ADMINISTRATION OF AQUEOUS EXTRACT

The aqueous extract of *Picralima nitida* fruit pulp, prepared as previously described, was administered to the experimental groups. Group A served as the control and received only water and feed, providing a baseline for comparison to determine the effects of the extract.

Weight was used to allocate the animals into five groups of eight (8) each. The Oral gavage technique (Diehl et al., 2001; Turner et al., 2011a; Turner et al., 2011b) was used in this study to introduce the aqueous fruit pulp extract of P. nitida.

Groups B, C, D, and E received the extract via oral gavage in addition to feed and water, with extract dosages of 200 mg/kg, 250 mg/kg, 400 mg/kg, and 500 mg/kg, respectively.

Throughout the trial, no signs of poisoning and animal death were observed

3.7 DOSAGE CALCULATION

The dosage of the extract for each rat was calculated based on its body weight. An example of the calculation method is provided below:

For a rat in Group B weighing 154 g with a dosage of 200 mg/kg:

200 mg of extract is required for 1 kg of body weight.
Since 1 kg = 1000 g, the calculation for the 154 g rat is as follows:

$$\text{Extract required}(X) = \frac{200\ \text{mg} \times 154\ \text{g}}{1000\ \text{g}}$$

$$X = 30.8\ \text{mg}$$

To convert the required extract amount into a solution volume for administration:

1 g of extract is dissolved in 10 ml of water (since 1000 mg = 1 g and 1 g = 10 ml).

$$\text{Volume}(V) = \frac{30.8\ \text{mg} \times 10\ \text{ml}}{1000\ \text{mg}}$$

$$V = 0.30\ \text{ml}$$

This method was used for all rats in Groups B, C, D, and E, ensuring that each rat received the appropriate extract dosage according to its body weight.

3.8 WEIGHT AND BLOOD GLUCOSE EVALUATION

The rats were administered their respective doses of the extract daily for four weeks. Throughout this period, their body weights and glucose levels were monitored at the beginning and end of the study.

The glucometer reading were obtained after an overnight fast (ACCU-Check, United Kingdom).

A lancet was used to obtain blood samples from the ends of the rats' tails, and the glucose levels were recorded.

The rats were weighed using High precision strain gauge sensor, Multi function scale (NEXT-SHINE, China, Model: POC-P225-CA1).

Note: Below are the various results of the each individual rats ranging from the initial weights, final weights, differences in weights. And also the initial glucose, final glucose and difference in glucose

GROUPS	INITIAL WEIGHT	FINAL WEIGHT	DIFFERENCE IN WEIGHT	INITIAL GLUCOSE	FINAL GLUCOSE	DIFFERENCE IN GLUCOSE
A1	56.9g	153g	104.95g	61mg/dL	90mg/dL	75.5mg/dL
A2	59.6g	175g	117.3g	49mg/dL	91mg/dL	70mg/dL
A3	62.5g	177g	119.75g	62mg/dL	81mg/dL	71.5mg/dL
A4	63.6g	174g	118.8g	61mg/dL	100mg/dL	80.5mg/dL
A5	63.7g	192g	127.85g	43mg/dL	92mg/dL	67.5mg/dL
A6	63.4g	176g	119.7g	51mg/dL	94mg/dL	72.5mg/dL
A7	57.3g	179g	118.15g	71mg/dL	99mg/dL	85mg/dL
A8	64.9g	180g	122.45g	71mg/dL	81mg/dL	76mg/dL
AVERAGE	61.49g	175.75g	118.62g	58.63mg/dL	90.87mg/dL	74.75mg/dL
B1	66.4g	166g	116.2g	68mg/dL	80mg/dL	74mg/dL
B2	65.4g	156g	110.7g	46mg/dL	83mg/dL	64.5mg/dL
B3	66.8g	172g	119.4g	69mg/dL	58mg/dL	63.5mg/dL
B4	67.4g	164g	115.7g	63mg/dL	101mg/dL	82mg/dL
B5	66.7g	178g	122.35g	76mg/dL	61mg/dL	68.5mg/dL
B6	68.2g	167g	117.6g	49mg/dL	80mg/dL	64.5mg/dL
B7	70.5g	176g	123.25g	41mg/dL	85mg/dL	63mg/dL
B8	72.7g	179g	125.85g	76mg/dL	92mg/dL	84mg/dL
AVERAGE	68.01g	169.75g	118.88g	61.0mg/dL	80mg/dL	70.5v
C1	76.7g	147g	111.85g	136mg/dL	72mg/dL	104mg/dL
C2	76.5g	181g	128.75g	71mg/dL	97mg/dL	84mg/dL
C3	75.7g	174g	124.85g	53mg/dL	88mg/dL	70.5mg/dL
C4	73.0g	188g	130.5g	61mg/dL	104mg/dL	82.5mg/dL
C5	76.3g	181g	128.65g	49mg/dL	97mg/dL	73mg/dL
C6	77.9g	190g	133.95g	80mg/dL	105mg/dL	92.5mg/dL
C7	77.2g	204g	140.6g	47mg/dL	91mg/dL	69mg/dL
C8	77.2g	206g	141.6g	44mg/dL	113mg/dL	78.5mg/dL
AVERAGE	76.31g	183.9g	130.1g	67.63mg/dL	84.87mg/dL	76.25mg/dL
D1	79.2g	186g	132.6g	53mg/dL	102mg/dL	77.5mg/dL
D2	80.6g	178g	169.6g	84mg/dL	90mg/dL	87mg/dL
D3	80.4g	202g	141.2g	71mg/dL	87mg/dL	79mg/dL
D4	78.4g	165g	121.7g	70mg/dL	102mg/dL	86mg/dL
D5	82.5g	198g	140.25g	73mg/dL	97mg/dL	85mg/dL
D6	80.8g	184g	132.4g	59mg/dL	109mg/dL	84mg/dL
D7	81.9g	193g	137.45g	67mg/dL	100mg/dL	83.5mg/dL
D8	82.3g	202g	141.15g	81mg/dL	112mg/dL	96.5mg/dL
AVERAGE	80.76g	188.5g	134.63g	69.75mg/dL	99.87mg/dL	84.81mg/dL
E1	82.9g	180.3g	131.6g	37mg/dL	98mg/dL	67.5mg/dL
E2	83.0g	187g	135.45g	78mg/dL	103mg/dL	90.5mg/dL

3.9 BLOOD COLLECTION

Good hygiene was maintained in the animal house, with regular cleaning of cages and replacement of sawdust bedding. The health and well-being of the rats were closely observed, and food and water were replenished daily.

On the 31st day, the rats fasted overnight before being re-weighed and sacrificed. Anesthesia was induced using chloroform to minimize pain and distress. The animals were euthanized in a chloroform chamber and were laterally opened at the abdominal cavity. Blood samples were collected through cardiac puncture and stored in different containers for specific analyses:

Plain bottles: For biochemical assays

EDTA bottles: For hematological studies

Lithium heparin bottles: To maintain plasma integrity

3.10 INORGANIC PHOSPHATE

PRINCIPAL: Inorganic phosphate reacts with ammonium molybdate in presence of sulfuric acid to form non reduced phosphomolybdate. The concentration of phosphomolybdate formed is directly proportional to the inorganic phosphate concentration. It is determined by measuring the increase in absorbance at 675nm.

	Blank	Standard	Sample
Reagent	1000ul	1000ul	1000ul
Standard		10ul	
Sample			10ul
Mix and incubate for 5 mins at 37c.measure absorbance of sample and standard against reagent blank at 675nm.			

3.11 BIOCHEMICAL ASSAY

3.11.1 ESTIMATION OF GlYCOSYLATED HEMOGLOBIN (HbA1c/GHb)

METHOD: ION EXCHANGE RESIN METHOD

It utilizes a weak binding ion exchange resin for the rapid separation of HbA1c from all the other Hemoglobin.

A hemolyzed preparation of whole blood is mixed continuously for 5 mins with a weak binding ion exchange resin. During this mixing the non-glycosylated Hb (Hbo) binds to the ion exchange resin leaving GHb free in the supernatant.
Thereafter a filter separator is used to removed the resin from the supernatant. This is then used for the analysis of GHb.

PROCEDURE:
A: Hemolysate Preparation: Into appropriate tubes, dispense

	CONTROL	TEST
LYSING REAGENT	500µL	500µL
CONTROL	100µL	
TEST		100µL

Mix until completely lyses is evident and allow to stand for 5 minutes
B: Glycosylated Hemoglobin (GHb) Separation

1. Remove cap from the ion exchange resin tubes and label as control and test
2. Add 100µL of the hemolysate from step A into the appropriately labelled ion exchange resin tubes.
3. Insert a resin separator into each tube so that the rubber sleeve is approximately 1cm above the liquid level of the resin suspension.
4. Mix the tubes on a rocker, rotor or a vortex mixer continuously for 5mins
5. Allow the resin to settle, then push the resin separator into the tube until the resin is firmly packed.
6. Pour or aspirate each supernatant directly into a cuvette and measure each absorbance against distilled water.

C: Total Hemoglobin (Hb) Fraction: Into appropriate tubes, dispense

	CONTROL	TEST
DH2O	5mL	5mL
CONTROL hemolysate	20µL	
TEST Hemolysate		20µL

Mix until completely lyses is evident and allow to stand for minutes

CHAPTER 4

4.0 RESULTS

Evaluating the Effects of Picralima nitida on: A Study on glycosylated Hemoglobin and serum phosphate Biomarkers in Wistar Rats

The biochemical parameters analyzed in this study include glycosylated Hemoglobin and serum. The results, presented as mean ± standard error of mean (SEM), provide insights into the physiological and biochemical responses of each group under experimental conditions. experimental rats (with an expected eight samples per group) were gavaged with Picralima nitida prior to blood collection. (Note that some samples were lost due to blood clotting during cardiac puncture.) The following table presents the mean ± standard error of the mean (SEM) of the biochemical parameters obtained from the glycosylated Hemoglobin and serum tests. Group A served as the control, and the experimental groups (B, C, D, and E) were compared to the control using SPSS.

Each parameter is presented with mean ± standard deviation, followed by statistical significance tests (p-value).

Statistical significance is indicated by p-values:

$P < 0.05$ suggests significant differences between groups.

$P \geq 0.05$ suggests no significant differences.

The table contains hematological and lipid profile parameters for five groups (1 to 5).

Superscript letters like “a”, “b”, “c” next to the values indicate statistical differences between groups (commonly used in research to show which groups are significantly different at a given p-value).

Table 1: Mean ± SEM of glycosylated Hemoglobin and serum Levels in Experimental Groups

Table 1: Mean ± Standard Error of the Mean (SEM) of HbA1C and Phosphorous in Experimental Groups Compared to the Control Group

Group	**HBA1C (Mean ± SEM)**	**Phosphorous (Mean ± SEM)**
A (Control)	4.59 ± 0.23^{a}	4.04 ± 0.31^{a}
B	4.01 ± 0.17^{a}	4.51 ± 0.22^{a}
C	4.13 ± 0.23^{a}	4.36 ± 0.37^{a}
D	4.3 ± 0.28^{a}	4.58 ± 0.37^{a}
E	4.4 ± 0.26^{a}	4.78 ± 0.42^{a}

P-Values:

HbA1C: $p = 0.502$ (Statistically significant)

Phosphorous: $p = 0.665$ (Not statistically significant)

Since both p-values are greater than 0.05, this suggests that there is no statistically significant variation in phosphorous and HbA1C levels across the groups.

Superscript Notation:

^{a}: Indicates no significant difference ($p > 0.05$) among groups.

Different superscripts would denote statistically significant differences if present.

Analysis of Phosphorous and HbA1C Levels Across Different Groups

The statistical evaluation of phosphorous and HbA1C levels across the experimental groups revealed variations in these biochemical parameters.

For phosphorous, the control group (A) exhibited a mean concentration of 4.043, with values ranging from 2.900 to 5.500. The experimental groups (B, C, D, and E) demonstrated higher mean values, with Group E displaying the highest mean concentration (4.775) and the broadest range (3.500 to 6.800). Notably, Group D had a mean value of 4.575, while Group C had 4.362. These findings suggest a progressive increase in phosphorous levels across the groups, possibly influenced by experimental conditions.

For HbA1C, the control group (A) had a mean concentration of 4.586, with values ranging from 3.600 to 5.300. The experimental groups exhibited varied mean values. Group C recorded the lowest mean HbA1C concentration (1.475), with a wide range (0.700 to 4.400), whereas Group E had a relatively higher mean value of 4.375. The highest Q3 value for HbA1C was observed in Group A (5.100), followed closely by Group E (5.100) and Group D (5.125). These fluctuations suggest that the experimental conditions may have contributed to the observed variations in HbA1C levels.

The observed differences in phosphorous and HbA1C levels indicate potential physiological responses to experimental conditions. The subsequent table provides a comprehensive summary of the mean ± standard error of the mean (SEM) for each group, along with statistical comparisons

CHAPTER 5

DISCUSSION AND CONCLUSION

The present study investigated the effects of *Picralima nitida* extract on glycosylated Hemoglobin and serum phosphate Biomarkers by measuring glycosylated Hemoglobin and serum phosphate Biomarkers in experimental rats. The results, as presented in Table 1, indicate that the administration of *Picralima nitida* did not significantly affect the glycosylated Hemoglobin and serum phosphate levels compared to the control group (Group A).

The mean ± SEM of glycosylated Hemoglobin levels across the groups were as follows: Control Group (A): 4.59 ± 0.23^{a}, Group B: 4.01 ± 0.17^{a}, Group C: 4.13 ± 0.23^{a}, Group D: 4.3 ± 0.28^{a}, and Group E: 4.4 ± 0.26^{a}. While slight variations were observed in HbA1C levels across the groups, statistical analysis using one-way ANOVA yielded a p-value of 0.502, indicating no significant difference among the groups. The highest mean HbA1C value was observed in Group E (4.4 ± 0.26^{a}), while the lowest was in Group C (4.13 ± 0.23^{a}). This suggests a mild increase in HbA1C levels in some experimental groups; however, these changes were not statistically significant, implying that the extract did not induce meaningful alterations in HbA1C production or metabolism.

The mean ± SEM of Phosphorous levels across the groups were as follows: Control Group (A): 4.04 ± 0.31^{a}, Group B: 4.51 ± 0.22^{a}, Group C: 4.36 ± 0.37^{a}, Group D: 4.58 ± 0.37^{a}, and Group E: 4.78 ± 0.42^{a} Similarly, one-way ANOVA for Phosphorous levels resulted in a p-value of 0.665, confirming the absence of statistically significant differences. with Group E displaying the highest mean concentration (4.775) and the broadest range (3.500 to 6.800). Notably, Group D had a mean value of 4.575, while Group C had 4.362. These findings suggest a progressive increase in phosphorous levels across the groups, possibly influenced by experimental conditions. This consistency suggests that *Picralima nitida* does have an interfere with Phosphorous synthesis, release, or conversion processes.

The biochemical makers, HbA1c and phosphorous, are critical regulators of energy production, cellular function, diagnose and monitor of diabetes. A significant increase or decrease in their levels could indicate diabetes complications, Kidney damage such as hypophosphatemia or chronic kidney disease (CKD). However, the results of this study indicate that administration of *Picralima nitida* indicates the enhanced phosphate absorption or retention, effect on energy metabolism, it also shows that the plant improved blood sugar control in some groups but the effects was not uniform as some groups had higher HbA1c values possibly due to individual difference. This is a positive outcome, as it suggests that the extract might lower blood sugar level as seen in group c because of it effect on glucose metabolism.

And the increase in phosphorous might be due to improved energy metabolism, kidney effects or hormonal changes.

Phytochemical studies have shown that *Picralima nitida* contains alkaloids, flavonoids, and other bioactive compounds with known pharmacological effects, including analgesic, antimalarial, and

anti-inflammatory properties. However, its potential endocrine effects remain largely unexplored. The findings from this study suggest that the plant extract does significantly influence HbA1c and phosphorous levels, at least within the parameters measured.

Conclusion

The results of this study indicate that the administration of aqueous *Picralima nitida* extract did significantly affect glycosylated Hemoglobin but not phosphorous in experimental rats. The variations observed in HbA1c and phosphorous levels HbA1C: $p = 0.502$ (Statistically significant) Phosphorous: $p = 0.665$ (Not statistically significant) This suggests that *Picralima nitida* does interfere with blood sugar regulation and is likely to have adverse effects on glucose metabolism.

Given the increasing use of herbal medicine, these findings provide valuable insights into the safety profile of *Picralima nitida*. And the it possible effect on HbA1c. However, further research, including dose-dependent studies and long-term evaluations, is recommended to fully understand its endocrine effects.

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