THE EFFECT OF PICRALIMA NITIDA AQUEOUS EXTRACT ON RENAL AND HEPATIC FUNCTIONS: A STUDY ON E/U/CREATININE AND LIVER FUNCTION BIOMARKERS IN ALBINO WISTAR RATS

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CERTIFICATION

This is to certify that this project work was carried out by **ERNEST CHIBUNDU KENNETH** with matriculation number **BMS2004992,** of the Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin City, in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc) degree in medical biochemistry.

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DEDICATION

I dedicate this work to Almighty God, my source of inspiration, strength, wisdom, knowledge and understanding and to the three most significant persons in my life, my father, mother and oldest sister. I don't say it or show it as I should but I really do mean it, Thank you.

ACKNOWLEDGMENT

As I reflect on my academic journey and the creation of this work, I am compelled to express my utmost gratitude to Almighty God for his guidance, wisdom and blessings that has shaped every word and thought in this work.

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ABSTRACT

Traditional medicine plays a crucial role in global healthcare, particularly in developing countries where plant-based remedies remain widely used. However, scientific validation of their safety and efficacy is necessary. This study examines the effects of a plant extract on liver and renal function in Wistar rats, focusing on its potential physiological impacts. Despite its traditional use, limited scientific data exist regarding its influence on hepatic and renal biomarkers.

This study investigates the impact of the extract on liver and kidney function in Wistar albino rats. A total of 40 rats were divided into control and experimental groups, with the test groups (B–E) receiving increasing doses of the extract, while Group A served as the control. The experiment monitored changes in body weight and evaluated liver function markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin levels. Additionally, renal function was assessed through creatinine, urea, and electrolyte levels. The study aimed to determine the extract's potential hepatotoxic or nephroprotective effects.

The findings of this study provide critical insights into the physiological effects of the plant extract, revealing its implications for liver and kidney health. The results contribute to the growing body of knowledge on the pharmacological properties of medicinal plants and their potential integration into modern therapeutic applications.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Medicinal plants have been widely used in traditional medicine for centuries, with many serving as sources of modern pharmaceutical compounds (Fabricant & Farnsworth, 2001). One such plant is Picralima nitida, commonly known as the Akuamma plant, which is native to West Africa (Iwu, 1993). It has been traditionally used for its analgesic, antimalarial, and anti-inflammatory properties (Olajide *et al.*, 2000). Various bioactive compounds, including alkaloids, have been identified in Picralima nitida, making it a subject of increasing scientific interest (Adotey *et al.,* 2012).

Serum creatinine and liver function tests (LFTs) are key biochemical parameters used to assess kidney and liver health, respectively (Nduka, 2006). Creatinine is a waste product generated from muscle metabolism and is primarily excreted by the kidneys. Its serum concentration serves as a marker of renal function (Waikar *et al.,* 2010). Similarly, liver function tests, which include enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and bilirubin levels, provide insight into hepatic integrity and function (Rosenthal *et al.,* 1999). Alterations in these parameters may indicate toxicity, dysfunction, or therapeutic effects of medicinal plants and other substances (Ozer *et al.*, 2008).

Given the increasing use of herbal remedies and the need to validate their safety and efficacy, it is crucial to investigate the potential impact of Picralima nitida on renal and hepatic functions (WHO, 2013). While some studies have suggested its pharmacological benefits (Akinmoladun et al., 2019), concerns remain regarding its possible toxicological effects on vital organs (Adotey *et al*., 2012). Therefore, this study seeks to Evaluate the Effects of Picralima nitida on Renal and Hepatic Functions after a controlled and observed period, providing scientific insights into its safety profile and potential therapeutic applications.

1.2 Statement of the Problem

Despite the long-standing use of Picralima nitida in traditional medicine for its purported analgesic, anti-inflammatory, and antimalarial properties, there is limited scientific evidence regarding its safety profile—particularly concerning its effects on vital organs such as the liver and kidneys. Anecdotal reports and preliminary studies suggest that while the plant may offer therapeutic benefits, its bioactive compounds could potentially induce adverse effects if consumed improperly or in high doses.

The lack of comprehensive studies that focus on critical biomarkers such as serum creatinine for renal function and various liver biomarkers (e.g., ALT, AST, ALP) and bilirubin level for hepatic functions creates an information gap. This gap poses significant challenges in validating the safety of Picralima nitida for therapeutic use, particularly given the increasing global reliance on herbal remedies.Therefore, this study seeks to address the following problems:

• Uncertainty in Safety Profile: There is insufficient data on whether Picralima nitida adversely affects kidney and liver functions.

• Lack of Dose-Response Information: The relationship between different dosages of Picralima nitida and the extent of its impact on E/U/Creatinine and liver function tests remains unclear.

• Implications for Therapeutic Use: Without clear evidence of its safety, the therapeutic application of Picralima nitida may pose unforeseen risks to patients, necessitating a thorough investigation.

By investigating the impact of Picralima nitida on serum creatinine and liver function tests in Wistar rats, this study aims to fill the existing gap in the literature, providing critical insights into its potential toxicological effects and informing safe usage guidelines for future medicinal applications.

1.3 Aim of the Study

This study aims to evaluate the Effects of picralima nitida extract on renal and hepatic functions of experimental wistar albino rats.

1.4 Objectives of the Study

The main objective of this study is to evaluate the impact of Picralima nitida on renal and hepatic functions in Wistar rats, thereby assessing its potential nephrotoxic and hepatotoxic effects. Specifically, this study aims to:

1. Assess Renal Function:

• Determine the effect of various doses of Picralima nitida on serum creatinine levels in Wistar rats.

• Investigate any indications of nephrotoxicity by comparing the serum creatinine levels of treated rats with those of the control group.

2. Evaluate Hepatic Function:

• Measure key liver enzymes, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP), as well as bilirubin levels, to assess liver function.

• Determine whether administration of Picralima nitida causes significant alterations in liver function tests indicative of hepatotoxicity.

3. To determine the concentration of serum level of electrolytes (sodium, potassium, bicarbonate, Chloride) Urea and creatinine

These objectives will help elucidate the potential risks associated with the use of Picralima nitida and contribute to the development of safer therapeutic practices involving this medicinal plant.

1.5. Scope of the Study

This research focuses on 40 male Wistar albino rats, divided into five groups, with 32 rats receiving Picralima nitida treatment over four weeks and eight serving as the control group. At the end of the study, blood samples were collected from all subjects to analyze electrolytes, Urea, and Creatinine levels and liver function markers, including ALT, AST, ALP and bilirubin level.. The study is limited to assessing biochemical markers without direct examination of the kidney or liver tissues.

1.6 Significance of the Study

The increasing global interest in herbal medicine necessitates scientific validation of medicinal plants to ensure their safety and efficacy. This study is significant in the following ways:

• Public Health Relevance: Findings from this study will provide critical insights into the safety profile of Picralima nitida, particularly regarding its potential renal and hepatic effects. This information is essential for both healthcare professionals and individuals using the plant for therapeutic purposes.

• Contribution to Pharmacology and Toxicology: By investigating changes in serum creatinine and liver function enzymes, this study contributes to the growing body of knowledge on herbal pharmacology and toxicology.

• Regulatory Implications: The results may inform regulatory bodies on the need for dosage guidelines and safety warnings regarding the prolonged use of Picralima nitida.

• Future Research Directions: The study will serve as a foundation for further investigations, including histological and molecular studies, to better understand the plant's effects on vital organs.

CHAPTER TWO

LITERATURE REVIEW

This chapter provides a comprehensive review of existing literature relevant to the study. It explores the botanical characteristics and pharmacological properties of Picralima nitida, as well as previous research on the impact of medicinal plants on renal and hepatic function in Wistar rats. The chapter also examines key biomarkers such as electrolytes/urea/creatinine

(E/U/Cr) and liver function parameters, highlighting their significance in assessing organ health. Additionally, it reviews the methodologies used in related studies and identifies gaps in the literature that justify the current research.

2.1 Overview of Pricalima nitida (Boanical description and pharmacology 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 includesthe 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 (Burkill, 1994; Oliver-Bever, 1986). 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 (Egharevba & Kunle, 2010; Okoye *et al*., 2014). These alkaloids have been found to interact with opioid receptors, contributing to the plant's potent analgesic and anti-inflammatory effects (Mbiantcha et al., 2018). 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 usedin the treatment of fever, malaria, and gastrointestinal disorders, while the bark and leaves are used for managing hypertension, dysentery, and inflammatory conditions (Akinpelu *et al.,* 2015). 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 (Gbedema *et al.*, 2006). 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 (Akinpelu et al., 2015). These biological effects are primarily attributed to the plant's rich bioactive compoundcomposition, 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 akuammicinemay 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.

2.2 Growth and Vegetative Characteristics

Picralima nitida typically manifests as a shrub or small tree, reaching heights of up to 15 meters. The plant thrives in humid, lowland forests and can also be found in disturbed sites, ranging from sea level up to elevations of 1,500 meters. Its leaves are broad, oblong, measuring between 6 to 20 centimeters in length and 3 to 10 centimeters in width, with 14 to 24 pairs of lateral nerves. The plant produces white flowers approximately 3 centimeters long. The fruits are ovoid, turning yellowish upon maturation.

2.3 Geographical Distribution

This species is native to Western Tropical Africa, extending eastward to Uganda. It is prevalent in countries such as Nigeria, Ghana, Cameroon, and Uganda. In Nigeria, it is known by various local names, including “Osu” in Edo, “Osi-Igwe” in Igbo, and “Abere” in Yoruba.

2.4 Medicinal Uses

Picralima nitida is extensively utilized in West African traditional medicine. Different parts of the plant such as the leaves, seeds, stem bark, and roots are employed to treat a variety of ailments, including fever, hypertension, jaundice, gastrointestinal disorders, inflammatory conditions (Iwu, 1993). Its seeds are commonly used as an analgesic, with reported effects similar to those of opioid analgesics (Abbiw, 1990).The plant contains indole alkaloids, which have been found to exhibit a broad range of pharmacological activities, lending credence to its ethnomedicinal uses.

2.5 Horticultural Uses

While Picralima nitida is primarily recognized for its medicinal properties, it also holds potential in horticulture. The plant's attractive white flowers and evergreen foliage make it suitable for ornamental purposes in gardens and landscapes. However, its use in horticulture is less documented compared to its medicinal applications

2.6 Bioactive Compounds

Phytochemical studies have identified several alkaloids in Picralima nitida, including akuammine, akuammidine, and picraline, which contribute to its pharmacological activities (Adotey et al., 2012). These alkaloids have been shown to exhibit analgesic, antipyretic, and anti-inflammatory properties (Osei-Safo *et al*., 2011). However, while these bioactive compounds offer therapeutic benefits, their potential toxicity to vital organs like the kidney and liver remains a concern, necessitating further toxicological evaluations.

2.7 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.

2.8 Overview of the Kidney(Structure and Function)

The kidneys are vital, bean-shaped organs located on either side of the spine, just below the rib cage. They play a crucial role in maintaining overall health by filtering waste products, balancing bodily fluids, and regulating various physiological processes.

2.8.1 Structure of the Kidney

Each kidney measures approximately 10–12 centimeters in length and weighs about 150 grams in adults. The internal structure of the kidney can be divided into several distinct regions:

1. Renal Cortex: The outermost layer, containing the glomeruli and convoluted tubules, is essential for filtering blood and initiating urine formation.

2. Renal Medulla: Located beneath the cortex, it consists of 8–12 cone-shaped renal pyramids. These pyramids contain the loops of Henle and collecting ducts, which concentrate urine.

3. Renal Pelvis: A funnel-shaped cavity that collects urine from the collecting ducts and channels it into the ureter.

4. Nephrons: The functional units of the kidney, each kidney contains approximately one million nephrons. Each nephron comprises a glomerulus and a renal tubule, working together to filter blood and form urine.

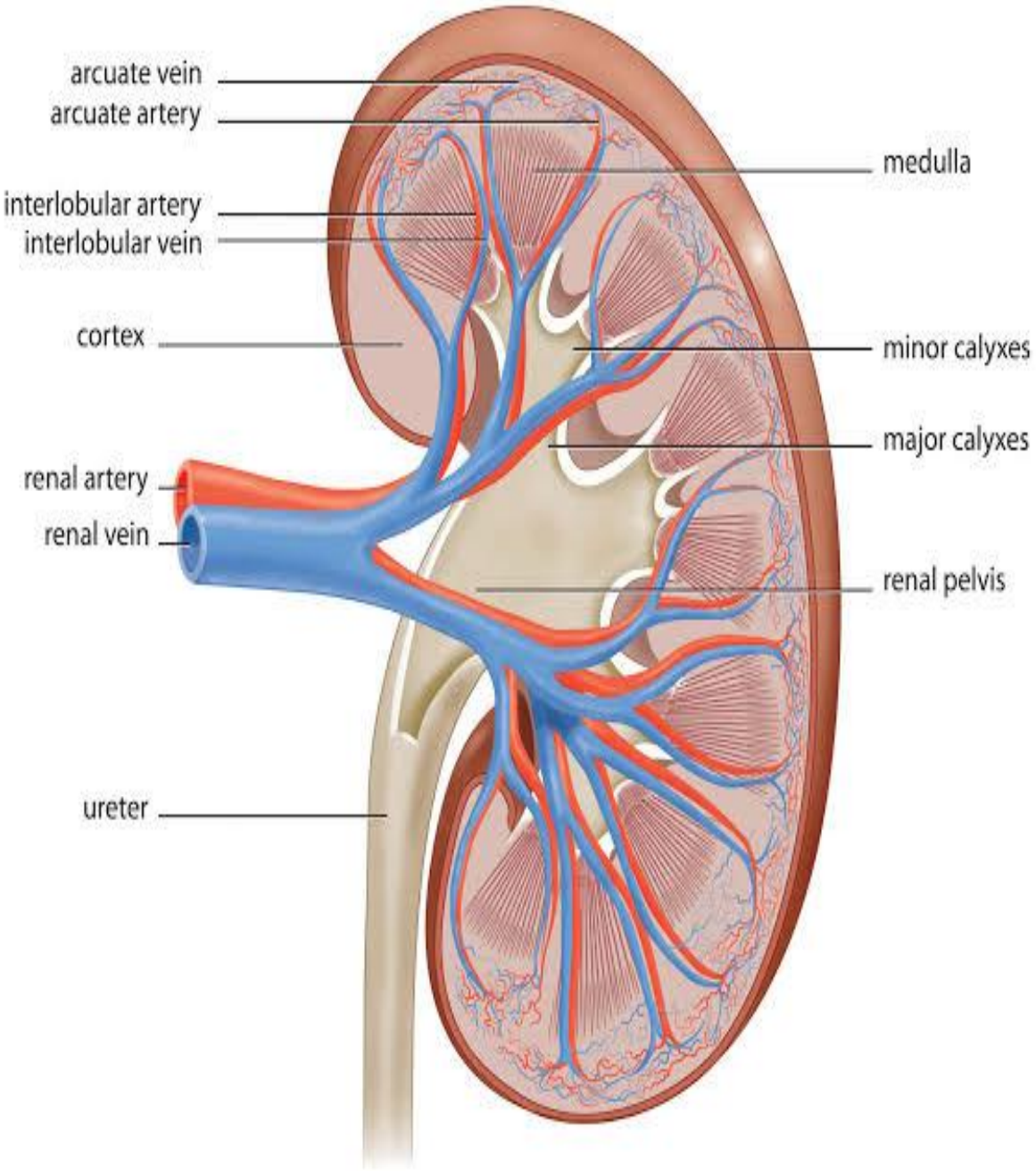


Figure 2.8.1

2.8.2 Functions of the Kidney

The kidneys perform several vital functions:

1 Filtration: Removing waste products and excess substances from the bloodstream to form urine.

2 Regulation: Maintaining electrolyte balance, blood pressure, and acid-base homeostasis.

3 Hormone Production: Synthesizing hormones like erythropoietin, which stimulates red blood cell production, and renin, which regulates blood pressure.

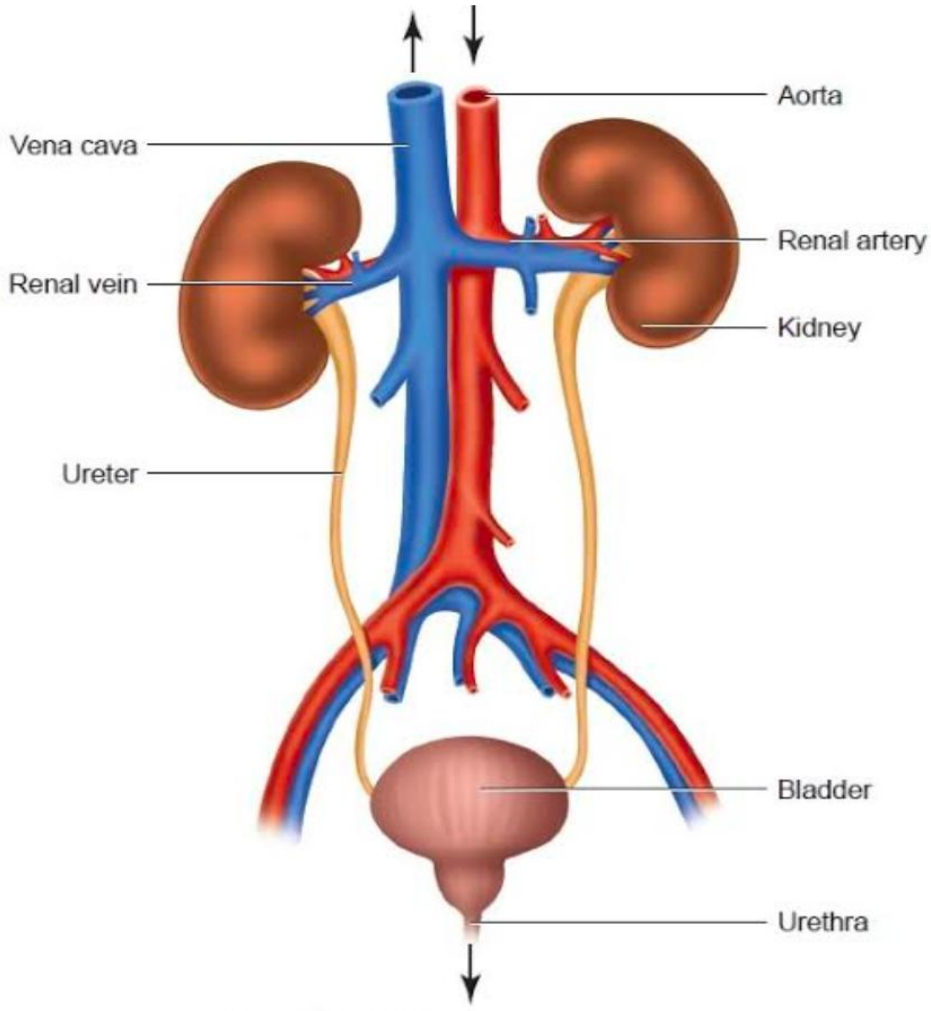


Figure 2.8.2

2.9 Overview of the Liver (Structure and Function)

The liver is a vital organ located in the upper right quadrant of the abdomen, beneath the diaphragm and above the stomach. It is the largest internal organ and gland in the human body, weighing approximately 1.4 kilograms in adults. The liver plays a crucial role in various metabolic processes, detoxification, and digestion.

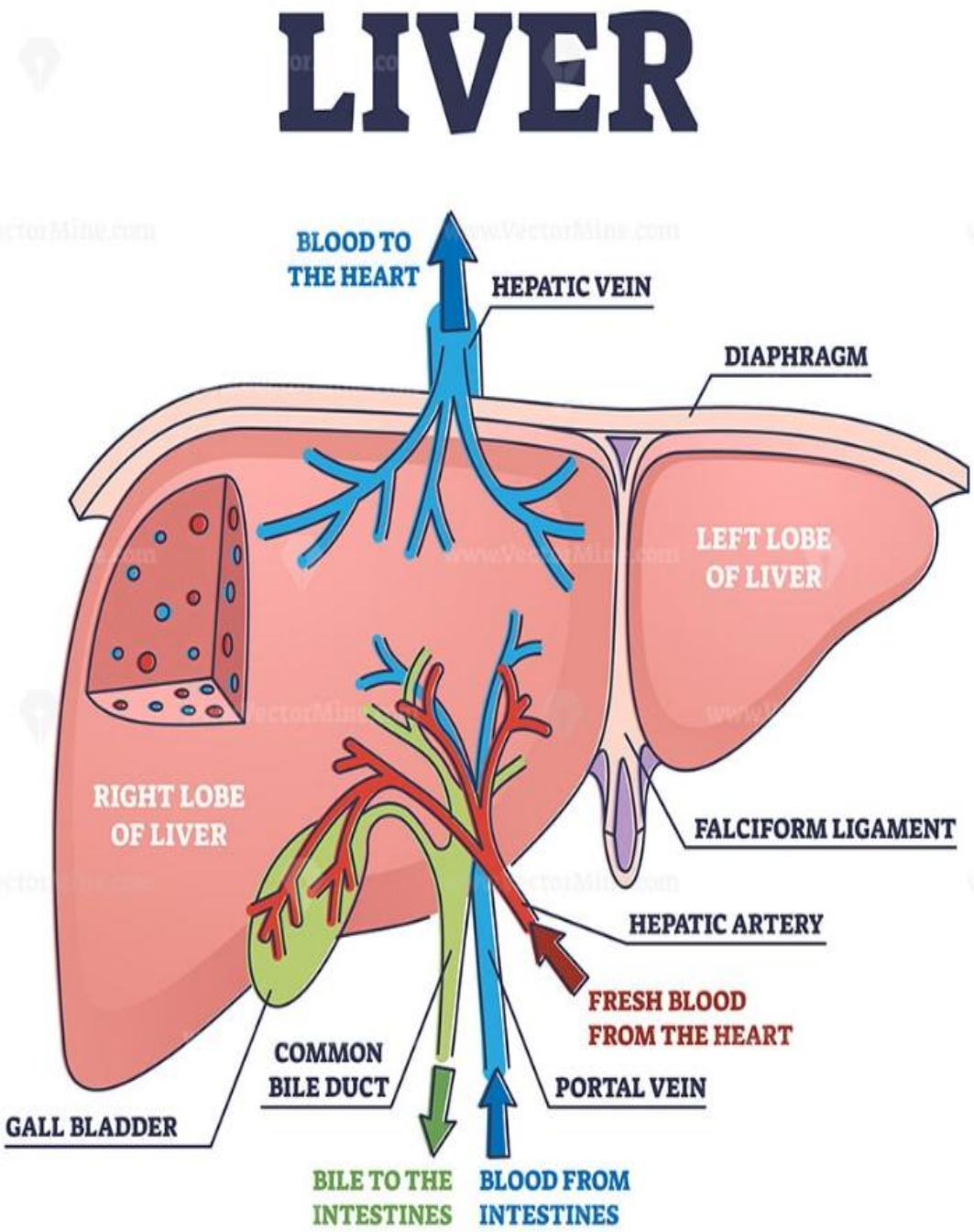
2.9.1 Structure of the Liver

The liver's anatomy is complex and can be described at both macroscopic and microscopic levels:

• Lobes: The liver is divided into two primary lobes—right and left—separated by the falciform ligament. The right lobe is larger and further subdivided into the caudate and quadrate lobes.

• Blood Supply: It receives oxygenated blood from the hepatic artery and nutrient-rich blood from the hepatic portal vein. This dual blood supply is essential for its metabolic and detoxifying functions.

• Lobules: Microscopically, the liver is composed of functional units called lobules. Each lobule consists of hepatocytes (liver cells) arranged in hexagonal patterns around a central vein. These structures facilitate efficient processing of blood and production of bile.



2.9.2 Functions of the Liver

The liver performs over 500 vital functions, including:

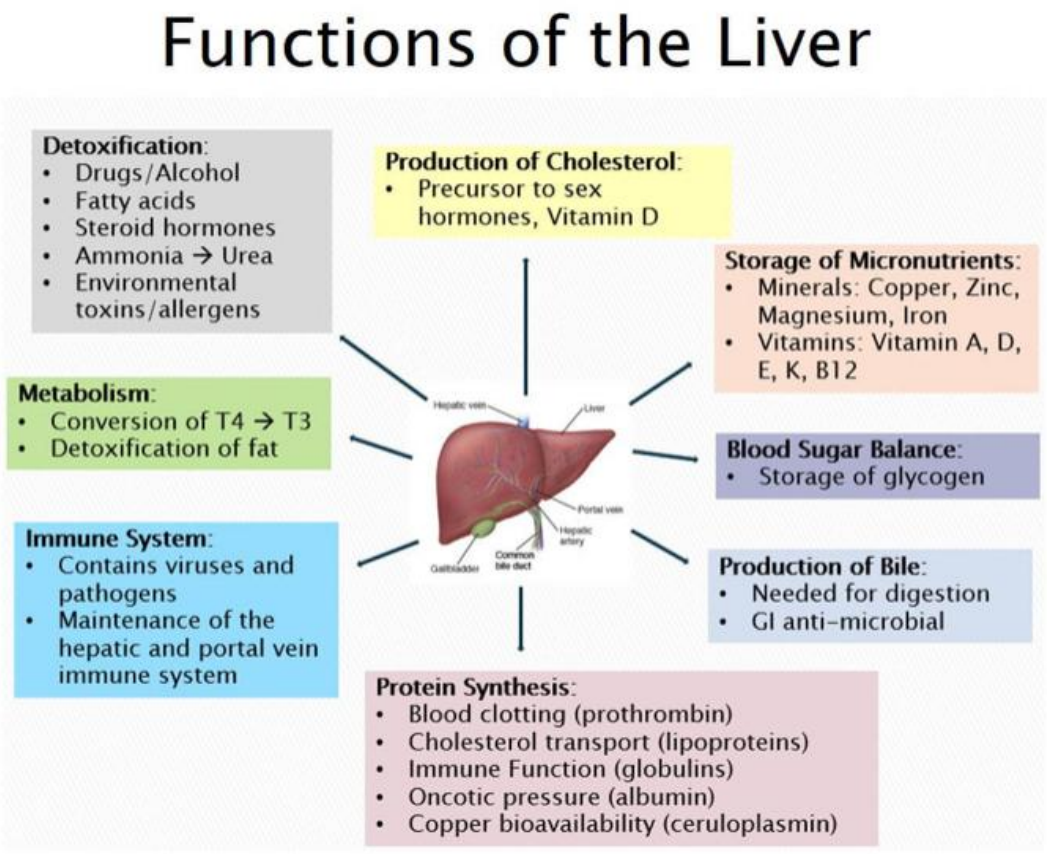
• Bile Production: It produces bile, which aids in the digestion and absorption of fats in the small intestine.

• Metabolism: The liver regulates carbohydrate, lipid, and protein metabolism. It maintains blood glucose levels by storing excess glucose as glycogen and releasing it when needed.

• Detoxification: It metabolizes and neutralizes toxins, drugs, and harmful substances, making them easier for the body to excrete.

• Synthesis of Proteins: The liver synthesizes essential proteins such as albumin, clotting factors, and enzymes vital for various bodily functions.

• Storage: It stores vitamins (A, D, E, K, and B12) and minerals like iron and copper, releasing them into the bloodstream as needed.



2.10 Effects of Medicinal Plants on Renal and Hepatic Function in Wistar Rats

Numerous studies have investigated the impact of medicinal plants on kidney and liver function using animal models, particularly Wistar rats. Herbal extracts rich in bioactive compounds have been shown to modulate renal and hepatic biomarkers, either by exerting protective effects or inducing toxicity. For example, studies on plants such as Vernonia amygdalina (Ezekwesili *et al.*, 2020), Moringa oleifera (Oyagbemi *et al.,* 2019), and Gongronema latifolium (Ugochukwu & Babady, 2021) have demonstrated their ability to enhance liver function and protect against nephrotoxicity induced by oxidative stress or toxic agents.

Research on Picralima nitida is relatively limited, but some studies suggest that its alkaloid-rich extracts possess potential nephroprotective and hepatoprotective effects (Akinmoladun et al., 2022). However, variations in extraction methods, dosages, and experimental models have led to inconsistencies in findings. While some reports indicate that Picralima nitida extracts improve renal and hepatic biomarkers (Ogunlana et al., 2020), others suggest potential toxicity at higher doses (Chinedu *et al.,* 2018). This discrepancy underscores the need for more systematic studies to clarify its effects on kidney and liver function.

2.11 Methodologies Used in Related Studies

Experimental studies on medicinal plants and their effects on renal and hepatic function typically involve in animal models using Wistar rats. These studies often employ biochemical assays to evaluate serum levels of creatinine, urea, electrolytes, ALT, AST, ALP, and bilirubin. Histopathological analysis of kidney and liver tissues is also commonly performed to assess morphological changes indicative of toxicity or protective effects.

Previous research has used various extraction techniques, including aqueous, ethanol, and methanol extracts, to isolate bioactive compounds from medicinal plants. The route of administration (oral or intraperitoneal) and the duration of treatment are critical factors influencing outcomes.

2.12 Effects of Picralima nitida on E/U/Cr biomarkers and LFT biomarkers

2.12.1 Effects on Kidney Function Biomarkers (E/U/Cr)

Research indicates that administration of ethanol seed extracts of Picralima nitida in rats led to significant increases in serum urea and creatinine levels compared to control groups. This suggests potential nephrotoxic effects at certain dosages.

2.12.2 Effects on Liver Function Test (LFT) Biomarkers

The impact of Picralima nitida on liver enzymes has been explored in various studies:

• A study evaluating the hepatoprotective properties of P. nitida extracts against alloxan-induced hepatotoxicity in rats found that administration of the extracts resulted in significant decreases in fasting blood glucose levels, as well as reductions in aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) levels. This suggests a protective effect on the liver.

• Conversely, another study reported that treatment with various doses of ethanol seed extract of P. nitida caused marked increases in serum levels of AST and ALT enzymes, indicating potential hepatotoxicity at higher concentrations (*OnlineScientificResearch.com*).

2.13 E/U/Creatinine as Indicators of Renal Health

Biomarker	Description	Significance
Electrolytes (E)	Includes sodium (Na^+), potassium (K^+), and chloride (Cl^-).	Imbalances may indicate impaired kidney function and fluid regulation.
Urea (U)	A waste product of protein metabolism.	Elevated levels suggest reduced kidney filtration efficiency or dehydration.
Creatinine (Cr)	A byproduct of muscle metabolism.	Increased serum creatinine levels indicate impaired glomerular filtration and potential renal dysfunction.

2.14 Liver Function Tests (LFTs) as Indicators of Hepatic Health

Biomarker	Function/Significance	Interpretation of Elevated Levels
Alanine aminotransferase (ALT)	Enzyme indicative of hepatocellular injury	Suggests liver cell damage (e.g., hepatitis)
Aspartate aminotransferase (AST)	Enzyme involved in amino acid metabolism	Indicates liver damage, but also found in muscles
Alkaline phosphatase (ALP)	Enzyme linked to bile duct function	May indicate cholestasis or bile duct obstruction
Bilirubin	Byproduct of hemoglobin breakdown	Suggests liver dysfunction or hemolysis

2.15 The Role of the Kidney and Liver in Maintaining Homeostasis

The kidneys and liver are vital organs involved in detoxification, metabolism, and maintaining homeostasis. The kidneys regulate electrolyte balance, remove metabolic waste products, and control blood pressure through the renin-angiotensin system. Creatinine, urea, and electrolytes (E/U/Cr) are critical biomarkers used to assess renal function. Increased serum creatinine and urea levels are often indicative of renal impairment, while electrolyte imbalances can signal disturbances in kidney function.

The liver, on the other hand, plays a central role in metabolism, protein synthesis, and detoxification. Liver function biomarkers such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin are essential for evaluating hepatic health. Elevated levels of these enzymes can indicate liver injury, hepatocellular damage, or cholestatic conditions.

2.16 Gaps in the Literature and Justification for the Study

Despite the growing interest in the medicinal potential of Picralima nitida, there are significant gaps in the literature regarding its effects on renal and hepatic function. Many studies have focused on its analgesic, antimicrobial, and antimalarial properties, with limited investigations into its impact on kidney and liver biomarkers. Additionally, inconsistencies in dosage, extraction methods, and experimental designs have made it challenging to draw definitive conclusions about its safety and efficacy.

This study aims to bridge these gaps by systematically evaluating the effects of Picralima nitida on renal and hepatic biomarkers in Wistar rats. By assessing E/U/Cr and liver function parameters, this research will provide valuable insights into the potential therapeutic or toxicological implications of Picralima nitida consumption. The findings could contribute to the scientific validation of its traditional use and guide its safe application in herbal medicine

CHAPTER THREE

MATERIALS AND METHODOLOGY

3.0 EUIPMENT AND APPARATUS:

- Hand Gloves
- Cotton wool
- Chloroform
- Dissecting set
- Methylated spirit
- Laboratory coat
- Sample container (Lithium heparin)
- Refrigerator (Hisense Refrigerator, Model; REF302DR)
- Weighing scale (NEXT-SHINE,china, POC-P225-CA1)
- Scissors
- Gavage
- Syringe (5ml and 10ml)
- Lancets
- Plain containers
- EDTA containers
- Knife

3.1 Machines

The following machines were used for the study

1. Glucometer (ACCU-Check, United Kingdom)
2. Weighing scale, (NEXT-SHINE, China, Model: POC-P225-CA1)
3. Storage system (Haier Thermocool chest freezer (Model: HTF-319H))
4. Grinder (SY-18B Industrial Dry Herbs Grinder)
5. Freeze Dryer (Biobase BK-FD10s Freeze Dryer (Xi’an, China))

3.2 ANIMAL EXPERIMENTAL STUDY

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.

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.

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.

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.

3.3 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.

3.4 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.

3.5 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 Picralima 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

3.6 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.

3.7 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
E3	122.5g	196g	159.25g	81mg/dL	98mg/dL	89.5mg/dL
E4	97.3g	193g	145.15g	71mg/dL	83mg/dL	77mg/dL
E5	85.3g	200g	142.65g	56mg/dL	96mg/dL	76mg/dL
E6	84.9g	203.6g	144.25g	72mg/dL	96mg/dL	84mg/dL
E7	85.6g	195g	140.3g	67mg/dL	98mg/dL	82.5mg/dL
E8	85.9g	206g	145.95g	53mg/dL	65mg/dL	59mg/dL
AVERAGE	90.93g	195.1g	143.01g	64.38mg/dL	92.12mg/dL	78.25mg/dL
TOTAL AVERAGE	75.5g	182.6g	129.05g	64.33mg/dL	89.55mg/dL	76.94mg/dL

3.8 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

The blood samples were stored at 4°C until analysis. Before biochemical analysis, the blood samples were centrifuged at 3000 rpm for 10 minutes to separate the serum. The collected serum was used to assess the serum levels of Electrolytes, Urea, and Creatinine (E/U/Cr) Test and also Liver Function Tests (LFTs) using standard biochemical techniques.

3.9 BIOCHEMICAL ASSAY

Biochemical assays are analytical techniques used to measure the concentration and activity of specific biomolecules in biological samples. In this study, biochemical assays were employed to assess kidney and liver function using Electrolytes, Urea, and Creatinine (E/U/Cr) Test and Liver Function Tests (LFTs). These assays provide valuable insights into metabolic and physiological conditions.

3.9.1 Estimation of Creatinine by Jaffe's Method

Principle: Creatinine in serum or plasma reacts with alkaline picrate to form a red-colored complex, which is measured spectrophotometrically at 500 nm. This method is widely used to assess kidney function.

Procedure:

• A working reagent was prepared by mixing alkaline solution and picric acid.

• 100 µL of serum sample was mixed with 1000 µL of creatinine working reagent and allowed to stand for 10 minutes.

• Absorbance was measured at 500 nm.

• The reference range for serum/plasma creatinine is 0.5 – 1.4 mg/dL.

3.9.2 Estimation of Urea by Urease/Berthelot Method

Principle: Urea in the sample is hydrolyzed by urease to form ammonia and carbon dioxide. The ammonia reacts with salicylate and hypochlorite in the presence of nitroprusside, forming a blue-colored indophenol complex, which is read at 550 nm.

Procedure:

• 100 µL of serum sample was mixed with urease enzyme and incubated at 37°C for 10 minutes.

• After adding color reagents, the mixture was incubated at 37°C for 15 minutes, then absorbance was read at 550 nm.

• The reference range for serum urea is 10 – 50 mg/dL.

3.9.3 Electrolyte Analysis

Electrolytes were analyzed using an Ion-Selective Electrode (ISE) Method. This technique is based on the potentiometric measurement of ions using selective membranes that allow for

rapid and precise quantification of sodium, potassium, bicarbonate, and chloride in serum samples.

Procedure:

1. Unhemolyzed serum samples were collected and stored at 2–8°C before analysis.

2. Samples were introduced into the ISE analyzer, which measures the ion activity using dedicated electrodes for each electrolyte.

3. The instrument automatically calculated the concentrations based on the Nernst equation.

4. Results were recorded and compared with reference ranges.

Reference Ranges:

• Sodium (Na^+): 135–145 mmol/L

• Potassium (K^+): 3.5–5.0 mmol/L

• Bicarbonate (HCO_3^-): 22–28 mmol/L

• Chloride (Cl^-): 96–106 mmol/L

3.9.4 Estimation of Alanine Aminotransferase (ALT)

Principle: ALT catalyzes the reaction between α-oxoglutarate and L-alanine, producing pyruvate and glutamate. Pyruvate reacts with 2,4-dinitrophenylhydrazine to form a colored complex, which is measured at 546 nm.

Procedure:

• 0.05 mL of serum sample was mixed with buffer solution (R1) and incubated at 37°C for 30 minutes.

• After adding color reagent (R2) and sodium hydroxide, absorbance was read at 546 nm.

• The reference range for ALT activity is up to 12 U/L.

3.9.5 Estimation of Aspartate Aminotransferase (AST)

Principle: AST catalyzes the conversion of L-aspartate and α-oxoglutarate to oxaloacetate and glutamate. The oxaloacetate hydrazine complex formed is measured at 546 nm.

Procedure:

• 0.05 mL of serum sample was incubated with buffer reagent at 37°C for 30 minutes.

• After adding color reagent and sodium hydroxide, absorbance was measured at 546 nm.

• The reference range for AST activity is up to 12 U/L.

3.9.6 Estimation of Alkaline Phosphatase (ALP) by Colorimetric Endpoint Method

Principle: ALP catalyzes the conversion of thymolphthalein monophosphate to thymolphthalein, which develops a blue chromogen in an alkaline medium and is measured at 590 nm.

Procedure:

• 0.5 mL of serum sample was incubated with substrate solution at 37°C for 3 minutes.

• The color developer was added, and the absorbance was read at 590 nm.

• The reference range for ALP activity is 9 – 35 U/L.

3.9.7 Albumin Estimation

Principle:

The estimation of albumin in serum is based on its quantitative binding to the bromocresol green (BCG) dye. Albumin reacts with BCG under acidic conditions to form a colored

complex, which exhibits maximum absorbance at 578 nm. The intensity of the color produced is directly proportional to the concentration of albumin in the sample.

Procedure:

1. Prepare serum samples ensuring they are unhemolyzed.

2. Pipette the following into test tubes:

• Standard: 0.01 mL of albumin standard + 3.00 mL of BCG reagent.

• Sample: 0.01 mL of serum sample + 3.00 mL of BCG reagent.

3. Mix thoroughly and incubate at room temperature (20–25°C) for 5 minutes.

4. Measure the absorbance of the sample and standard at 578 nm using a spectrophotometer.

5. Calculate the albumin concentration using the formula:

$$\left(\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard}\right) * Concentration\ of\ standard$$

3.9.8 Total Protein Estimation

Principle:

The Biuret method is based on the reaction of cupric ions (Cu^{2+}) in an alkaline medium with peptide bonds in proteins, forming a violet-colored complex. The intensity of the color, measured at 546 nm, is directly proportional to the protein concentration in the sample.

Procedure:

1. Collect unhemolyzed serum samples and store at 2–8°C before analysis.

2. Prepare the following reaction mixtures:

• Blank: 0.02 mL distilled water + 1.0 mL Biuret reagent.

• Standard: 0.02 mL of total protein standard + 1.0 mL Biuret reagent.

• Sample: 0.02 mL of serum sample + 1.0 mL Biuret reagent.

3. Mix well and incubate at room temperature (20–25°C) for 30 minutes.

4. Measure absorbance at 546 nm using a spectrophotometer.

5. Calculate total protein concentration using the formula:

$$\left(\frac{Absorbance\ of\ sample}{Absorbance\ of\ standard}\right) * Concentration\ of\ standard$$

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CHAPTER 4

4.1.0 Evaluating the Effects of Picralima nitida on Renal and Hepatic Function: A Study on E/U Creatinine and Liver Function Biomarkers in Wistar Rats

The biochemical parameters analyzed in this study include urea, sodium (Na^+), potassium (K^+), creatinine, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) across five experimental groups (A, B, C, D, and E). 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 E/U/Cr and liver function biomarker tests.
Group A served as the control, and the experimental groups (B, C, D, and E) were compared to the control using SPSS. One-way ANOVA was conducted to determine statistical significance, followed by Tukey's HSD Post Hoc Test to assess pairwise comparisons between groups.

The statistical analysis ($p < 0.05$) performed using SPSS validates these differences, providing a robust basis for interpreting the impact of Picralima nitida on the biochemical profile of the treated rats. Tukey's HSD Post Hoc Test was applied to determine which specific groups showed significant differences in biochemical parameters. Different superscripts (a, b) indicate statistically significant differences ($p < 0.05$).

4.2 Mean ± SEM of Renal function parameters of control and test (administered aqueous extract of P.nitida fruit pulp) albino wistar rats

Param	A (Ctrl)	B	C	D	E
U (mg/dL)	28.14 ± 1.20[a]	29.63 ± 1.06[a]	28.75 ± 1.10[a]	28.25 ± 1.15[a]	29.88 ± 1.50[b]
Na (mmol/L)	140.00 ± 1.50[a]	137.63 ± 1.60[b]	141.63 ± 1.55[a]	146.38 ± 1.70[a]	141.88 ± 1.65[a]
K (mmol/L)	5.93 ± 0.15[a]	5.86 ± 0.20[a]	5.78 ± 0.18[a]	6.14 ± 0.22[a]	5.60 ± 0.20[b]
HCO_3 (mmol/L)	20.57 ± 0.80[a]	21.50 ± 0.85[a]	21.25 ± 0.90[a]	20.38 ± 0.80[a]	22.50 ± 0.95[b]
Cl (mmol/L)	105.14 ± 2.00[a]	103.88 ± 1.90[a]	102.50 ± 1.85[a]	102.88 ± 2.00[a]	104.50 ± 1.95[a]
Cr (mg/dL)	0.54 ± 0.05[a]	0.58 ± 0.04[a]	0.59 ± 0.05[a]	0.63 ± 0.05[a]	0.65 ± 0.05[b]

Table 4.2

4.3 Mean ± SEM of Hepatic function parameters of control and test (administered aqueous extract of P.nitida fruit pulp) albino wistar rats

Parameters	A (Control)	B	C	D	E
AST (U/L)	22.00 ± 1.80^{a}	24.38 ± 1.70^{a}	26.25 ± 2.00^{a}	22.88 ± 1.90^{a}	22.63 ± 1.80^{a}
ALT (U/L)	9.43 ± 0.90^{a}	9.75 ± 0.85^{a}	11.88 ± 1.00^{b}	10.00 ± 0.95^{a}	10.75 ± 0.95^{a}
ALP (U/L)	33.29 ± 1.50^{a}	30.38 ± 1.40^{a}	32.88 ± 1.55^{a}	32.13 ± 1.45^{a}	34.63 ± 1.60^{a}
CB (mg/dL)	0.37 ± 0.05^{a}	0.35 ± 0.04^{a}	0.33 ± 0.04^{a}	0.26 ± 0.03^{b}	0.28 ± 0.03^{b}
TB (mg/dL)	0.83 ± 0.05^{a}	0.73 ± 0.04^{a}	0.70 ± 0.05^{a}	0.63 ± 0.04^{b}	0.56 ± 0.04^{b}
TP (g/dL)	6.76 ± 0.10^{a}	6.74 ± 0.10^{a}	7.00 ± 0.11^{a}	6.66 ± 0.10^{a}	6.54 ± 0.10^{a}
ALB (g/dL)	3.50 ± 0.10^{a}	3.48 ± 0.10^{a}	3.59 ± 0.10^{a}	3.49 ± 0.10^{a}	3.31 ± 0.10^{b}

Table 4.3

In this study, the p-value threshold was set at **$p < 0.05$**, meaning that a p-value less than 0.05 indicates a statistically significant difference between groups. Superscripts (e.g., a, b) were used to denote these differences:

- **Same superscripts** (e.g., 'a' in urea levels of groups A, B, C, and D) indicate **no significant difference** among these groups.
- **Different superscripts** (e.g., 'b' in group E for urea) indicate a **significant difference** compared to groups with the 'a' superscript.

Key observations of Picralima nitida extract in Renal function parameters

1. **Urea (U) Levels (mg/dL)**

Observations:

- Slight fluctuations, but no significant increase or decrease

Reasons:

- Since urea is a waste product of protein metabolism excreted by the kidneys, stable levels suggest no major renal impairment.

2. Sodium (Na) Levels (mmol/L)

Observations:

- Sodium slightly decreased in B but increased in C, D, and E, peaking in D.

Reasons:

- The rise in sodium at higher doses (D, E) suggests a potential effect of the extract on sodium retention or electrolyte balance.

3. Potassium (K) Levels (mmol/L)

Observations:

- K levels remained fairly stable across all groups

Reasons:

- Potassium is crucial for nerve and muscle function. Since levels did not change drastically, the extract likely did not significantly impact potassium excretion.

4. Bicarbonate (HCO_3^-) Levels (mmol/L)

Observations:

- A slight increase in group E was observed

Reasons:

The slight increase in E could indicate that the extract might influence pH regulation at higher doses.

5. Chloride (Cl^-) Levels (mmol/L)

Observations:

- A slight decrease in B, C, and D followed by a return to near-control levels in E.

Reasons:

- Chloride plays a key role in maintaining fluid balance, osmolarity, and acid-base homeostasis.
- The drop in chloride might indicate temporary changes in kidney filtration and reabsorption, possibly as a compensatory response to other electrolyte shifts

6. Creatinine (Cr) Levels (mg/dL)

Observations:

- Creatinine increased progressively from B to E, with the highest value in E.

Reasons:

- Creatinine is a key marker of kidney function. A gradual increase suggests a mild reduction in renal clearance at higher doses.

However, since the increase is not drastic, it may not indicate severe kidney dysfunction but rather a mild strain on renal filtration due to higher metabolic demands or extract components.

General interpretation of effects of picralima nitida fruit pulp extract on Renal functions in wistar Albino Rats

The assessment of renal function in this study was based on key biochemical parameters, including creatinine, urea, and electrolyte levels (Na^+, K^+, Cl^-, HCO_3^-). The results indicate that while the extract influenced kidney function, there is no strong evidence of severe renal damage. However, some metabolic adjustments were observed, particularly at higher doses.

Considering the changes observed in creatinine, urea, and electrolytes, the extract appears to have induced some metabolic changes in renal function but did not cause severe nephrotoxicity. The gradual increase in creatinine suggests that higher doses may place additional strain on the kidneys, but since urea remained stable and electrolyte imbalances were mild, the kidneys were still functioning properly.

Further studies, such as histopathological analysis of kidney tissues, could provide additional confirmation of these findings.

Key observations of picralima nitida extract on Hepatic function parameters

1. **AST (Aspartate Aminotransferase) Levels**

Observation:

- A mild increase in B and C, then a return to near-control levels in D and E.

Reasons:

- AST is involved in amino acid metabolism and liver function. The initial rise in B and C might indicate mild hepatocellular stress or adaptation to the extract.
- The return to near-control levels in D and E, despite a higher dose, could suggest tolerance development or a protective effect at higher doses.

2. **ALT (Alanine Aminotransferase) Levels**

Observation:

- Increased in all test groups, highest in C.

Possible Reason:

- ALT is more liver-specific than AST. A rise indicates possible hepatocellular activity or mild stress.
- Since group C had the highest ALT level while E had a moderate increase despite the highest dose, this could mean a threshold effect, where mild stress occurs at moderate doses (C), but the liver adapts at higher doses (D, E).

3. **ALP (Alkaline Phosphatase) Levels**

Observation:

- ALP remained relatively stable across groups, with minor fluctuations.

Possible Reasons:

- Since ALP is linked to bile duct function and bone metabolism, no significant changes suggest the extract did not impair bile flow or cause liver damage.
- The slight fluctuations could be due to metabolic adaptation rather than toxicity.

4. **CB (Conjugated Bilirubin) and TB (Total Bilirubin) Levels**

Observations:

- CB decreased in D and E.
- TB showed a dose-dependent decrease, with the lowest level in E.

Possible Reasons:

- Enhanced bilirubin clearance or improved liver detoxification at higher doses.
- A hepatoprotective effect could be in play, where the extract helps the liver process bilirubin more efficiently as dose increases.

5 TP (Total Protein) and ALB (Albumin) Levels

Observations:

- TP slightly increased in C, but gradually reduced in D and E.
- ALB remained stable but showed a minor drop in E.

Possible Reasons:

- The increase in C might be due to a metabolic boost from moderate doses of the extract.
- ALB levels is stable across all groups, Since albumin is produced by the liver, its stability suggests no significant damage.

General interpretation of effects of picralima nitida fruit pulp extract on Hepatic functions in wistar Albino Rats

The extract of P. nitida did not cause liver dysfunction but led to mild metabolic stress at moderate doses (C), followed by possible liver adaptation at higher doses (D, E). The decrease in bilirubin levels and stability of albumin suggest potential hepatoprotective effects, while enzyme fluctuations indicate temporary adjustments rather than liver injury.

Thus, there is no clear evidence of liver toxicity, but further studies, such as histopathological analysis, could help confirm these findings.

Overall, these statistically significant differences—indicated by the assigned superscripts—suggest that the oral administration (via gavage) of Picralima nitida affects certain renal and hepatic biomarkers. In particular, the observed elevations in urea and creatinine and the alterations in bicarbonate and albumin in Group E warrant further investigation into the dose-dependent effects of this herbal extract. The statistical analysis ($p < 0.05$) performed using SPSS validates these differences, providing a robust basis for interpreting the impact of Picralima nitida on the biochemical profile of the treated rats.

CHAPTER FIVE

DISCUSSION, SUMMARY, FINDINGS, CONCLUSIONS, AND RECOMMENDATIONS

5.1 Discussion

This study examined the effects of Picralima nitida fruit pulp extract on renal and hepatic function in Wistar rats by analyzing key biochemical parameters such as urea, creatinine, electrolytes (Na^+, K^+, Cl^-, HCO_3^-), and liver function biomarkers (AST, ALT, ALP, bilirubin, and total protein). The findings were compared across control (Group A) and test groups (B, C, D, E) to determine dose-dependent changes.

5.1.1 Effects on Renal Function

The renal biomarkers analyzed in this study provide insight into kidney function and the possible nephrotoxic effects of Picralima nitida.

• Creatinine levels progressively increased from B to E, peaking in Group E, suggesting a mild strain on renal clearance at higher doses. Although creatinine levels remained within physiological limits, this trend indicates that excessive consumption of Picralima nitida may impose a metabolic burden on the kidneys.

• Urea levels showed no significant variation across groups, indicating that protein metabolism and kidney filtration efficiency were not substantially affected. The stability of urea suggests that renal impairment, if present, was mild.

• Electrolyte balance fluctuated slightly, with sodium levels increasing at higher doses (D, E), while chloride levels decreased in B, C, and D before normalizing in E. These changes suggest that the extract may influence kidney function by altering electrolyte reabsorption or fluid homeostasis.

These findings suggest that Picralima nitida extract induces mild metabolic stress on renal function but does not cause severe nephrotoxicity. However, prolonged or excessive use could pose risks, particularly for individuals with pre-existing kidney conditions.

5.1.2 Effects on Hepatic Function

The hepatic biomarkers measured in this study assessed the impact of Picralima nitida on liver function.

• ALT increased in all test groups, peaking in C, before slightly declining in D and E. Since ALT is a liver-specific enzyme, its elevation in the early test groups suggests mild hepatocellular stress. However, the decline at higher doses (D, E) may indicate an adaptive response of the liver to the extract.

• AST showed a mild increase in B and C but returned to near-control levels in D and E. This suggests that while there was initial hepatic stress, the liver may have adapted to the extract at higher doses.

• ALP remained relatively stable across all groups, indicating that the extract did not significantly affect bile duct function. This stability suggests that hepatobiliary function was not impaired.

• Bilirubin levels (total and conjugated) decreased in D and E, suggesting improved bilirubin clearance or a hepatoprotective effect of the extract at higher doses.

• Total protein increased slightly in C but declined in D and E, while albumin levels remained stable. This suggests that the extract influenced protein metabolism but did not cause significant liver dysfunction.

Overall, these findings suggest that Picralima nitida does not induce severe hepatotoxicity. Instead, it appears to cause mild hepatic stress at moderate doses, followed by an adaptive or protective effect at higher doses.

5.1.3 Comparison with Existing Literature

Previous research on Picralima nitida has reported conflicting findings, with some studies suggesting hepatoprotective and nephroprotective effects, while others indicate potential toxicity at high doses. The findings of this study align with both perspectives:

• The observed increase in creatinine and ALT at moderate doses suggests mild metabolic stress, similar to studies reporting toxicity at higher doses (Chinedu *et al.,* 2018).

• The decline in bilirubin and stable ALP levels at higher doses align with reports that Picralima nitida may have hepatoprotective effects (Okoye *et al.*, 2014).

These findings suggest that Picralima nitida exhibits dose-dependent effects, where moderate consumption is tolerable, but excessive intake could lead to metabolic strain on the liver and kidneys.

5.2 Summary

This study assessed the effects of Picralima nitida fruit pulp extract on renal and hepatic function in Wistar rats. Forty male rats were divided into five groups: a control group (A) and four test groups (B–E) receiving increasing doses of the extract. Biochemical markers, including creatinine, urea, electrolytes, ALT, AST, ALP, and bilirubin, were measured to determine potential nephrotoxic or hepatotoxic effects.

The findings revealed that while the extract induced mild metabolic changes in kidney and liver function, there was no strong evidence of severe toxicity. Creatinine levels increased at higher doses, suggesting mild renal strain, while ALT levels rose at moderate doses before stabilizing at higher doses, indicating hepatic adaptation. Electrolyte balance was slightly affected, but bilirubin clearance improved, suggesting a potential protective effect.

These results highlight the importance of dose regulation when using Picralima nitida in traditional medicine and suggest that while moderate consumption may be safe, excessive intake could pose risks.

5.3 Findings

The key findings of this study are:

1. Renal Function Findings:

• Creatinine levels increased progressively, indicating mild renal strain at higher doses.

• Urea levels remained stable, suggesting no significant impairment in kidney function.

• Electrolyte fluctuations were observed, with increased sodium and decreased chloride levels in some groups.

2. Hepatic Function Findings:

• ALT levels increased at moderate doses, indicating transient hepatic stress.

• AST levels fluctuated but normalized at higher doses, suggesting liver adaptation.

• ALP remained stable, indicating that bile duct function was not impaired.

• Bilirubin clearance improved at higher doses, suggesting a potential hepatoprotective effect.

3. General Findings:

• The extract exhibited dose-dependent effects, where moderate doses caused mild metabolic stress, but higher doses led to adaptive responses.

• No severe nephrotoxicity or hepatotoxicity was observed, but prolonged or excessive use could pose risks.

5.4 Conclusion

This study provides valuable insights into the impact of Picralima nitida on renal and hepatic function. The findings suggest that while the extract induces mild metabolic changes, it does not cause severe toxicity. The increase in creatinine at higher doses suggests potential renal strain, while the changes in ALT and bilirubin indicate transient hepatic stress followed by adaptation.

These results underscore the importance of controlled consumption of Picralima nitida in herbal medicine. While the extract may have therapeutic benefits, particularly in bilirubin clearance, excessive use could impose metabolic stress on vital organs.

Further studies, including histological analysis and molecular investigations, are necessary to confirm these biochemical findings and determine the long-term safety profile of Picralima nitida.

5.5 Recommendations

Based on the findings of this study, the following recommendations are made:

5.5.1 Future Research

• Histological Studies: Microscopic analysis of kidney and liver tissues should be conducted to confirm structural changes.

• Molecular Mechanisms: Research should focus on identifying the bioactive compounds responsible for observed biochemical effects.

• Long-term Studies: Chronic toxicity studies should assess the long-term impact of Picralima nitida consumption.

5.5.2 Safe Usage Guidelines

• Moderate Consumption: Users should limit intake to avoid potential renal or hepatic stress.

• Standardized Dosages: Regulatory bodies should establish safe dosage guidelines for medicinal use.

• Monitoring in Herbal Medicine: Traditional medicine practitioners should be informed of potential dose-dependent effects.

5.5.3 Public Health and Policy Recommendations

• Awareness campaigns should educate the public on the potential risks of excessive Picralima nitida consumption.

• Government agencies should support further research to establish safety regulations for herbal products.

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