PHYTOCHEMICAL INVESTIGATION AND BIOCHEMICAL EFFECT OF METHANOL ROOT EXTRACT OF *PICRALIMA NITIDA*

BY

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SCHOOL OF PHARMACY

UNIVERSITY OF BENIN

BENIN CITY

APRIL, 2024

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A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, FACULTY OF PHARMACY, UNIVERSITY OF BENIN, BENIN CITY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF DOCTOR OF PHARMACY DEGREE HONOURS IN PHARMACY.

APRIL, 2024

CERTIFICATION

This is to certify that this work was done by Ebiuwa Egbon in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, Benin city.

_____	_____
Dr. Osayemwenre Erharuyi	**Date**
(Supervisor)	
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Dr. Imieje Vincent	**Date**
(Head of Department)	
_____	_____
Egbon Ebiuwa	**Date**
(Student)	

DEDICATION

This work is dedicated to everyone that believes in and is currently using herbal medicine.

Also, to Ijabila. For believing that I can write.

ACKNOWLEDGEMENTS

I would like to thank God for giving my life purpose and for seeing me through the period of this project. I am grateful to him for life, strength, wisdom and grace.

I would also like to thank my supervisor, Dr. Osayemwenre Erharuyi, for always being available to answer questions, point out corrections and for all his help during the entire time.

I want to specially thank my mother, Mrs Evbayekha for her kind words, hugs and financial support. I would not have made it this far without you. Thank you, ma.

I am grateful for my family, for being shoulders to lean on. For the laughter, hugs and everything in between. You people are the sun to my moon. I would not shine without you.

My Peculiar. If the whole world was in chaos, you would always show up with clarity mixed with laughter and joy. Thank you for listening to me talk about this project all day. We did it!

Special thanks to Owa, Joan, Peace and Sandra. Sisters who will always have your back. Thank you for helping me through getting this degree.

I am grateful to my local church, for understanding my many moments of absenteeism and to pastor, for every word spoken and prayed over.

Special shout out to Peter, for your office space, laptop and for allowing me evade your personal space, thank you!

Finally, all my project colleagues, Samuel, Igiebor, Uyi, Happiness and Edith, thank you for being here, we did it guys! We really really did it!

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ABSTRACT

Picralima nitida, commonly known as Akuamma, is a plant native to West Africa and has been traditionally used for its medicinal properties.

This study aims to investigate the phytochemical composition and subchronic toxicity profile, with a specific focus on biochemical analysis and histopathology of rats administered the roots of *Picralima nitida*.

Phytochemical screening was carried out using standard chemical tests and the subchronic toxicity study was done for 28 days with the administration of the methanolic root extract to the rats.

Biochemical analysis and histopathological studies were carried out after to assess subchronic toxicity effects.

Picralima nitida was shown to contain phytochemicals most likely responsible for its pharmacological properties and therefore, its ethnomedicinal uses. The rats in the treatment groups based on the biochemical parameters showed no signs of toxicity when compared to the control group.

In conlusion, phytochemical screening and subchronic toxicity evaluation are essential steps in assessing the safety and efficacy of medicinal plants. The study demonstrates that the root extract at doses administered were not toxic. However, further studies are needed where the animals are given higher doses with longer durations to fully understand the plant's toxicity.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction.

Picralima nitida has been used for so many years for the treatment of various ailments

The roots of *Picralima nitida* have been traditionally used for various therapeutic purposes, including the treatment of gastrointestinal disorders, and respiratory ailments, and as a natural aphrodisiac.

According to the World Health Organization (WHO), traditional medicine serves as a primary therapeutic option for 80% of the global population residing in emerging nations. In recent years, there has been a noticeable surge in the utilization of complementary and alternative medicine (CAM), notably herbal remedies, even in developed countries (Mukherjee, 2002).

In Africa, the widespread use of ethnomedicine persists despite the availability of alternative treatments. This enduring reliance on traditional medicine can be attributed to two key factors. Firstly, inadequate access to Western medical treatments and medications due to factors such as geographical remoteness, prohibitive costs, or limited healthcare provider availability. Secondly, certain diseases, such as malaria and HIV/AIDS, remain prevalent in Africa, where effective modern medical treatments for these conditions are lacking compared to other regions (Fawzi, 2021).

Despite the common perception that herbal medicines are natural and therefore presumed to be devoid of toxicity or adverse effects, it has simply not been true based on research done.

It is important to acknowledge that some may contain a complex array of up to 200 chemicals. This intricate chemical composition poses challenges in assessing the safety of herbal drugs. While evaluating the activity and potential side effects of a single chemical compound may be

relatively straightforward, comprehending the interactions and synergistic effects among multiple chemicals within a plant or crude plant extract presents a more intricate task (Philomena, 2011).

Phytochemical screening, the process of analyzing plant extracts for the presence of bioactive compounds, is an essential step in understanding the pharmacological potential of medicinal plants. *Picralima nitida* is known to contain a diverse array of phytochemicals, including alkaloids, flavonoids, tannins, saponins, and terpenoids, (Awodele *et al.,*2019) which are believed to contribute to its medicinal properties. However, a comprehensive phytochemical analysis of *Picralima nitida* roots is needed to elucidate its chemical composition and to fully understand its pharmacological activities.

Subchronic toxicity studies are conducted to evaluate the potential adverse effects of plant extracts when administered repeatedly over a relatively short period. While *Picralima nitida* has been traditionally used for its therapeutic benefits, limited scientific evidence exists regarding its safety profile, particularly concerning the subchronic toxicity of the roots of the plant.

By combining phytochemical analysis with toxicity assessment, this study seeks to provide information concerning the safety and toxicity of the roots of *Picralima nitida.*

1.2 Medicinal plant

Traditional medicine has existed with mankind since the very beginning of time. Plants have been a source of food, clothing, shelter, and medicines since the start of time. (Ameeenah, 2006). Medicinal plants are plants that contain compounds with therapeutic properties, which can be used to prevent, alleviate, or cure various ailments and health conditions.

Traditional medicine (TM), also known as ethno-medicine, folk medicine, native healing, or complementary and alternative medicine (CAM), is an ancient and culture-bound method of healing that humans have used to cope and deal with various diseases that have threatened their existence and survival. (Abdullahi, 2011)

According to the World Health Organisation, TM is "the sum total of the knowledge, skills, and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness" (WHO, 2002b).

1.2.1 Medicinal Plants as sources of natural products

Herbal medicine encompasses traditional medicinal practices and therapeutic innovations passed down through generations. (Sofowora and Ogunbodede, 2013).

A medicinal plant is characterized by containing substances within its organs that are utilized for therapeutic purposes or serve as precursors for drug synthesis. The global market value of medicinal plant products exceeds $100 billion annually, reflecting their widespread use and significance. Evidence from fossils indicates that humans have relied on plant-based remedies for at least 60,000 years. (Shi *et al.,*2010)

These medicinal plants are often utilized in the form of crude drugs, involving dried parts such as roots, stems, bark, leaves, and fruits, or their extracts. The study of medicinal plants has always been important due to their role in preventing, treating, and managing various health conditions, ranging from acute illnesses like malaria and dysentery to chronic diseases such as diabetes and hypertension. Remarkably, over 25% of drugs in modern Pharmacopeias are derived from medicinal plants, highlighting their pharmaceutical importance. (Daswani *et al.,* 2006)

Many medicinal plants have served as sources of lead compounds in drug discovery processes. For example, in the 19th century, the purification of essential medications like morphine, digitoxin, and quinine, which continue to be widely used today was done. (Balunas and Kinghorn, 2005). Across diverse societies, medicinal plants form the foundation of complex traditional medicine systems, fulfilling basic healthcare needs.

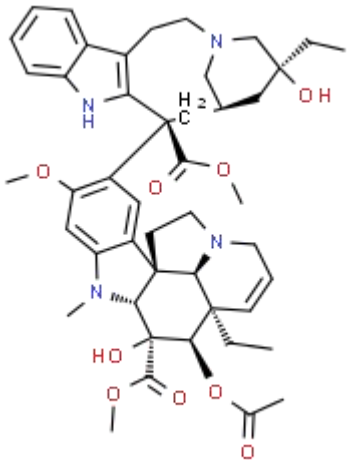
Today, there is growing concern regarding the efficacy, purity, and safety of herbal medications, both in developed and developing nations. However, traditional medicines offer potential pathways for future healthcare system development, with opportunities to extract and analyze active ingredients from medicinal plants. (Fatemah *et al.,* 2018). Using the knowledge of traditional medicine and medicinal plants presents a promising avenue for discovering and utilizing natural plant products.

Natural products encompass a diverse array of small biological molecules produced by living organisms, ranging from entire organisms to specific plant parts like leaves, stems, fruits, and exudates, as well as their primary and secondary metabolites. Today, a significant proportion of conventional medications manufactured by developed nations are derived from natural sources, underscoring the importance of natural products in modern pharmacotherapy. In fact,

approximately 60% of such medications can be traced back to natural origins. (Liu and Wang, 2007). Ongoing research and clinical development efforts continue to unveil new natural products with therapeutic potential, particularly in the realm of anti-neoplastic agents. (Nwonu *et al.,* 2019).

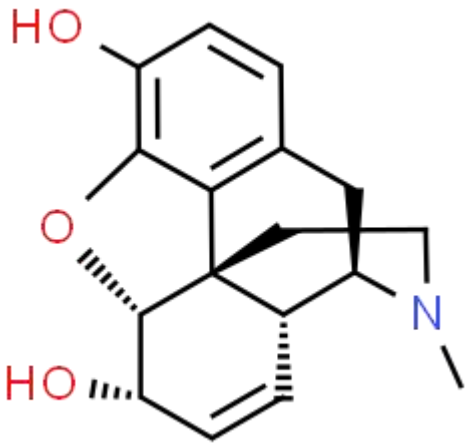
The process of isolating, identifying, and purifying natural products from medicinal plants has yielded numerous notable discoveries throughout history. Examples include the extraction of morphine, strychnine, quinine, caffeine, nicotine, cocaine, atropine, and mixtures of cardiac glycosides from various plant sources. (Hussein, 2019). Notably, vinca alkaloids like vincristine and vinblastine, which are crucial in the management of testicular carcinoma and Hodgkin's disease, are sourced from the Madagascan Periwinkle. Natural products offer a vast reservoir of chemical diversity and bioactivity, making them invaluable resources for drug discovery and development. (Harvey, 2008). Their molecular structures often possess unique pharmacological properties that can be harnessed for therapeutic purposes. Also, natural products derived from medicinal plants often come with a long history of traditional use, providing a foundation for further scientific investigation and validation of their efficacy and safety.

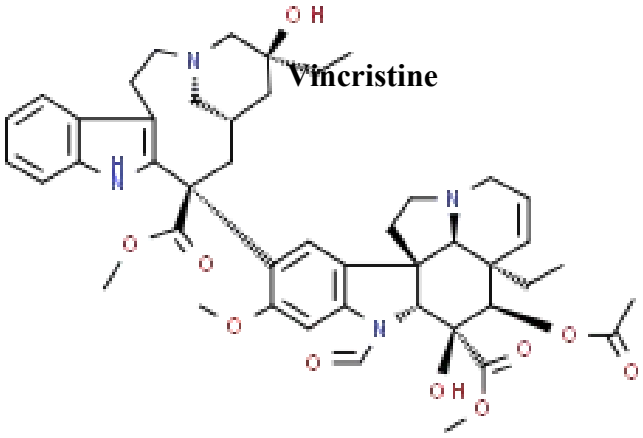
The figure below shows structures of drugs derived from medicinal plants.



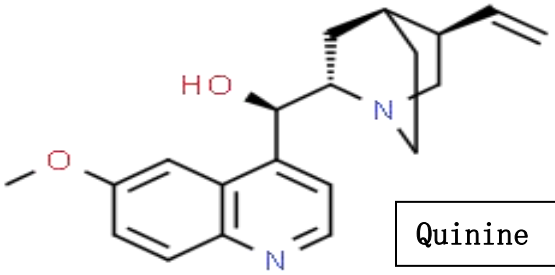
Vinblastine

Morphine

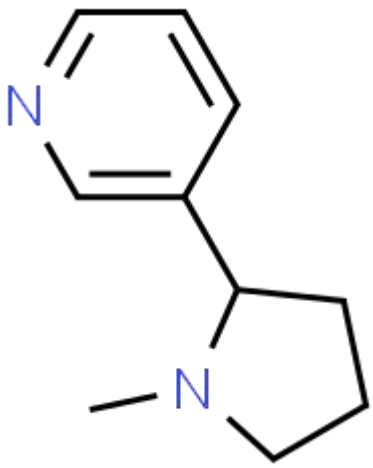




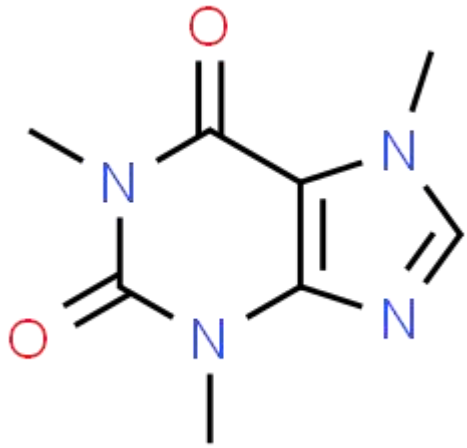
Vincristine



Quinine



Nicotine



Caffeine

Figure 1.1 Structures of drugs derived from natural products

1.3 West African Medicine

Medicinal plants play a vital role in traditional healthcare systems throughout West Africa, benefiting from the region's diverse plant species. For centuries, these plants have been utilized

to address various ailments(Cambridge University Press, 1986) In contrast to Western medicine, traditional African medicine adopts a holistic approach rooted in the interconnectedness of human beings. This approach often incorporates indigenous herbalism into treatment methods.

According to traditional African beliefs, individuals consist of several interconnected parts, including physical, spiritual, moral, and social aspects. When these elements function harmoniously, a person experiences good health. (Ancient Origins, 2023)

West African medicine has been around for a long time and has come to stay. Understanding the herbs used, and regulating the methods of preparation and ways of administration will allow the proper integration of West African medicine with Western medicine.

1.3.1 Brief History

Before the advent of Western medicine, traditional medicine served as the primary healthcare system for millions of people across Africa, spanning rural and urban communities alike (Romero-Daza, 2002).

The history of medicinal plants in West Africa is deeply intertwined with the region's traditional healthcare systems. For centuries, these plants have played a crucial role in African medicine, utilized for purposes such as prevention, diagnosis, improvement, and treatment of a wide array of physical and mental illnesses (WAHO, 2020).

1.3.2 Safety and Claims

Some medicinal plants in the past have been pushed by people who believed that such plants are capable of curing every kind of disease (Schumaker, 2012).

Although medicinal plants provide numerous health benefits, it's crucial to recognize that they also pose risks. Certain plants inherently contain toxins, and if used incorrectly, they can result in adverse reactions. Also, issues like herb-drug interactions, poor adherence to good manufacturing practices, inadequate regulatory oversight, and the presence of adulterants can all undermine the safety of herbal medicines (Mensah *et al.,* 2019). These factors collectively contribute to a range of adverse effects, spanning from mild to severe. Hence, it highlights the necessity for careful and knowledgeable utilization of medicinal plants.

One of the main issues is the limited toxicological data available for many herbal medicines, despite their long history of use. For instance, a study on the root extracts of *Pueraria lobata* and *Scutellaria baicalensis* highlighted the need for more comprehensive data, as these herbs have been traditionally used in East Asia to treat various conditions but lacked extensive toxicological evaluation (Song, 2020)

1.3.3 Toxicity of Herbal Medicines

Research involving in vivo assessments of aqueous extracts generally support the safety of herbal medicines. However, findings from in vitro tests conducted on isolated individual cells frequently yield different results. This emphasizes the importance of conducting toxicity studies that accurately reflect traditional usage patterns.

Such studies are essential for facilitating informed discussions regarding the safety of herbal remedies. It is imperative to ensure that toxicity assessments align with historical and cultural practices to provide a rational and comprehensive understanding of safety considerations associated with medicinal plant usage. (Mensah *et al.,*2019)

1.4.Toxicity

Toxicity is the measure of a substance's ability to cause harmful effects in living organisms (Mensah *et al.,* 2019). It can also be defined as the degree of damage inflicted by a chemical substance on exposed tissues, encompassing effects on entire organisms as well as their cellular (cytotoxicity) or organ (organotoxicity) components. Toxicity encompasses the study of adverse chemical effects on living organisms, including their symptoms, underlying mechanisms, and potential treatments. Depending on the amount and duration of exposure to agents, toxicity studies are categorized into acute, subacute/subchronic, and chronic effects (Denny *et al.,*2013)

1.4.1 Acute toxicity

Acute toxicity refers to the harmful effects that occur in a short period of time, (about 24 hours) usually following a single or large dose exposure to a substance.

The results of acute toxicity are not only important in the consideration of accidental poisoning with a chemical but also are used for the planning of chronic toxicological studies. (Herxheimer, 1987)

The starting point for the toxicological classification of chemicals uses the LD50 value, which is the dose administered in acute toxicity testing that causes death in 50% of experimental animals (WHO, 2010)

1.4.3 Chronic toxicity

Chronic toxicity refers to the adverse effects of a substance that occur after prolonged or repeated exposure over a longer duration, which can range from weeks to years.

Chronic toxicity studies focus on the long-term health effects of exposure to a substance. These effects can include organ damage, reproductive harm, and increased risk of cancer (Sahil *et al.,* 2021*)*

The results of chronic and acute toxicological studies help in the evaluation of any possible hazardous effect of a new drug or a drug that is in use with little or no documentation of its systemic toxicity. (Mensah *et al.,* 2019)

Periods between acute and chronic exposure could be referred to as subacute or subchronic.

1.4.4 Subchronic Toxicity

Subchronic toxicity studies involve studying the harmful effect of a substance from repeated exposure occurring typically after 28 to 90 days. These studies monitor various health parameters, including body weight, food and water intake, organ weights, blood biochemistry, and histopathological examinations of vital organs to detect any signs of toxicity (Tauheed *et.al,* 2021)

The results from subchronic toxicity studies are used to assess the risk of long-term exposure to medicinal plants and to establish safe dosage levels for human use. (Tauheed *et.al,* 2021)

1.5 *Picralima nitida*

Picralima Stapf) T. Durand and H. Durand (Apocynaceae) is a shrub or a tree and it is a popular medicinal herb in Africa, where it is commonly gathered from the wild for local medicinal use. (Tropical Plant Database, 2014). In Nigerian traditional medicine, *Picralima nitida* is highly regarded for its diverse medicinal uses

1.5.1 Scientific Classification

Kingdom Plantae

Phylum Streptophyta

Class Equisetopsida

Subclass Magnoliidae

Order Gentianales

Family Apocynaceae

Genus *Picralima*

Species *Picralima nitida*

International Plant Names Index and World Checklist of Vascular Plants (2024)

1.5.2 Plant Description

The Akuamma plant, known scientifically as *Picralima nitida* is a glabrous tree or shrub, 9-75 ft. high with white to yellow flowers in terminal, mostly densely contracted, inflorescences.

This species is native to the forests of Africa, ranging from the Ivory Coast to Uganda. It features white flowers approximately 3 cm in length and produces ovoid fruits that turn yellowish when ripe. The plant's leaves are broad and oblong, measuring between 3-10 cm in width and 6-20 cm in length, with 14 to 24 pairs of sturdy, small lateral nerves. (NNMDA, 2008)

The wood is hard with pale yellow and a cylindrical shape. The diameter of the trunk ranges from 5 to 60 meters (Okonta and Aguwa, 2007).

1.5.3 Geographical Distribution

The plant is widely distributed in high deciduous forests of West-Central Africa from Ivory Coast to West Cameroons and extending across the Congo Basin and Uganda.

According to (Adjanohoun *et al.,* 1996), the distribution range of P. nitida extends from Côte d'Ivoire to Uganda including the Democratic Republic of Congo and Cabinda region in Angola.

The plant is native to Benin, Cabinda, Cameroon, Central African Republic, Congo, Gabon, Ghana, Guinea, Ivory Coast, Nigeria, Uganda, Zaïre. (POWO, 2024)

1.5.4 Common names

Akuamma plant

Other Nigerian names

Igbo- òsú igwe (Obasi, 2012)

Yoruba -Abeere

Edo- Osu (Duweijua,2002)

1.5.5 Ethnomedicine of *Picralima Nitida*

Various parts of the plant *Picralima nitida* are utilized in preparing herbal mixtures to treat or prevent a number of ailments. Renowned for its bitter taste, all parts of the plant, including the seeds, bark, and roots, are esteemed for their medicinal properties. Traditionally, the seeds, bark, and roots have been esteemed for their antipyretic properties, serving as remedies for malaria, pain relief, and alleviating chest and stomach problems, pneumonia, and intestinal worms (Burkil, 2004).

The seeds, despite their bitterness, are commonly ingested, sometimes with lemon juice, to alleviate conditions such as hernias, vomiting, and diarrhea. They are also utilized as a substitute for quinine in managing fevers. In powdered form, the seeds are employed to address pneumonia and other respiratory issues. Topically, crushed seeds are applied as a remedy for abscesses, while a mixture of ground seeds and shea butter is used to treat leucorrhoea in women. However, it's important to note that the seeds can be toxic in certain regions of Africa, restricting their use primarily to external applications (Erahuyi *et al.,* 2014).

In regions like Gabon and Ghana, *Picralima nitida* seeds are utilized for treating abscesses through topical application or oral administration. The seeds are crushed and consumed orally to address gastrointestinal problems, pneumonia, and chest complaints, or administered as a decoction enema (Erahuyi *et al.,* 2014).

The leaves of *Picralima nitida* serve as a vermifuge and are applied topically for otitis (Iwu, 1993). Additionally, the bark of the plant is employed as a laxative, purgative, antihelminthic, and for treating various ailments including venereal diseases, fever, and hernia. It is commonly crushed, chewed, or decocted for consumption, often sweetened with sugar, to address conditions such as fevers, coughs, male sterility, jaundice, yellow fever, food poisoning, and internal parasites (Useful Tropical Plants Database, 2014).

Research has demonstrated the efficacy of *Picralima nitida* bark against gram-positive bacteria and fungi, as well as its effectiveness against *Trypanosoma brucei,* the causative agent of sleeping sickness. Furthermore, the seed extract exhibits a rapid blood sugar-lowering effect compared to conventional medication (Useful Tropical Plants Database, 2014).

Regarding the root, *Picralima nitida* is valued for its vermifuge, aphrodisiac, fever-reducing, and anti-malarial properties, as well as its effectiveness in managing pneumonia and gastrointestinal disorders (Awedele *et al.,* 2019).

Additionally, *Picralima nitida* is utilized in the treatment of erectile dysfunction, female infertility, and in preventing nausea and vomiting during the first trimester of pregnancy (Erharuyi *et al.,* 2014).

1.6 Phytochemical screening

1.6.1 Phytochemicals

Phytochemicals, also known as phytonutrients, are natural compounds found in plants (De Silva *et al.,* 2017). These substances, categorized as secondary metabolites, can be synthesized through altered synthetic pathways from primary metabolites or share substrates originating from primary metabolites (Kabera, 2014). Phytochemicals encompass a wide range of compounds, including

alkaloids, flavonoids, tannins, phenolics, saponins, steroids, glycosides, and terpenes. Although not classified as essential nutrients like vitamins and minerals, phytochemicals play crucial roles in plant growth, development, and defense mechanisms.

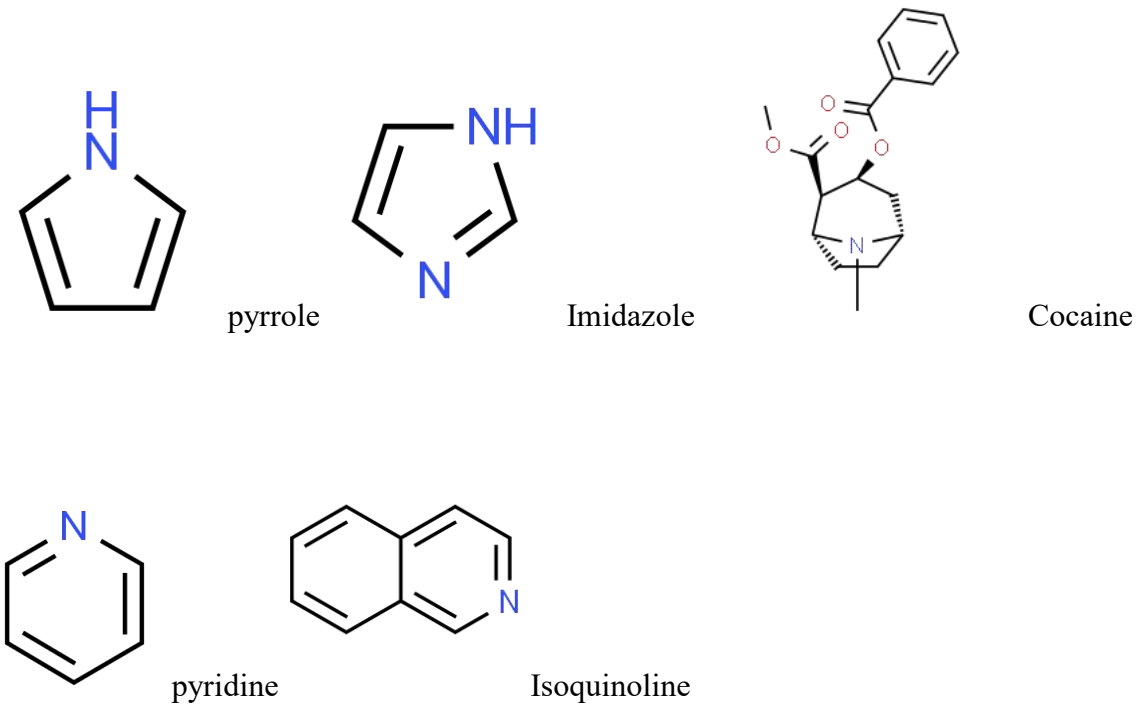
Natural products, which include phytochemicals, possess diverse biological properties and find applications across various sectors such as medicine, insecticides, herbicides, perfumes, and dyes. Secondary metabolites, which are synthesized by plants, are typically produced during specific growth stages or in response to environmental stressors (Avalos, 2009). These metabolites serve vital functions in plant defense against predators, pathogens, and stressors, as well as in reproductive processes like attracting insects for pollination.

Over the years, extensive research has helped us see the beneficial properties of phytochemicals, generating increased interest in their potential health-enhancing effects in humans.

1.6.2 Common classes of Phytochemicals

1. **Alkaloids:** Alkaloids are nitrogen-containing compounds known for their pharmacological properties, such as analgesic, anti-inflammatory, and antimalarial effects (De Luca *et al.,* 2012). Examples of alkaloids include caffeine, nicotine, and morphine.
2. **Flavonoids:** Flavonoids are polyphenolic compounds with antioxidant and anti-inflammatory properties. They are found abundantly in fruits, vegetables, and beverages like tea and wine (Panche *et al.,* 2016). Quercetin, kaempferol, and epigallocatechin gallate (EGCG) are examples of flavonoids known for their health-promoting effects.
3. **Tannins:** Tannins are polyphenolic compounds that contribute to the astringent taste of certain foods and beverages. They have antioxidant and antimicrobial properties and are found in foods like tea, grapes, and nuts (Rahman *et al.,* 2020).

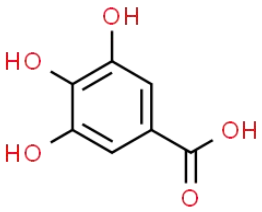
4. **Phenolics:** Phenolics are aromatic compounds with antioxidant properties. They are widely distributed in plant foods and have been associated with various health benefits, including reducing the risk of chronic diseases (Liu, 2013).
5. **Saponins:** Saponins are glycosides with foaming properties and are found in various plants, particularly legumes and herbs. They have been studied for their cholesterol-lowering, anticancer, and immunomodulatory effects (Sedighi *et al.,* 2019).
6. **Terpenes:** Terpenes are a diverse class of compounds derived from isoprene units and are found in essential oils of plants. They exhibit a wide range of biological activities, including antimicrobial, anti-inflammatory, and anticancer properties (Newman and Cragg, 2012)



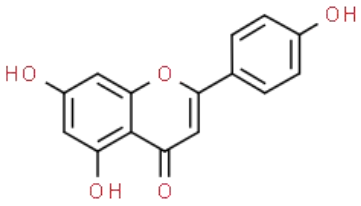
pyrrole Imidazole Cocaine

pyridine Isoquinoline

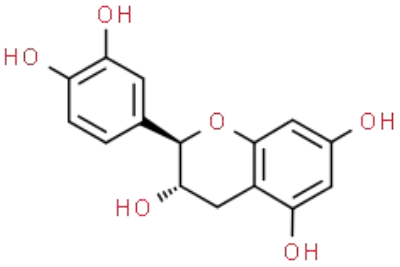
Figure 1.2: Skeletal structures of alkaloids found in medicinal plants.



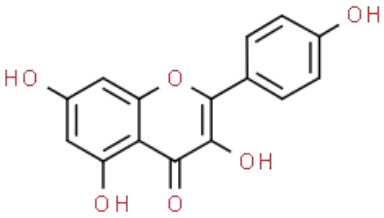
Gallic acid



Apigenin

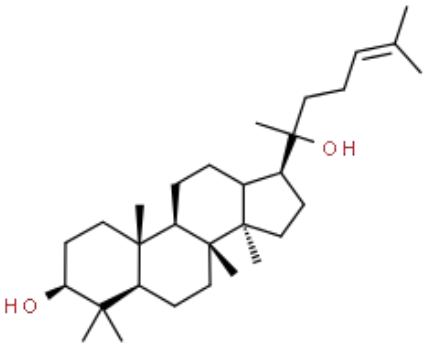


Catechin

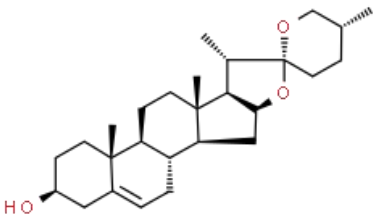


Kaempferol

Figure 1:3 Skeletal structures of some phenolic compounds in plants

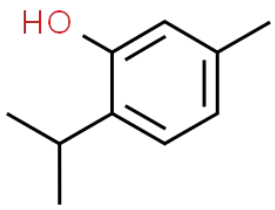


Ginsenosides

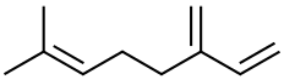


Diosgenin

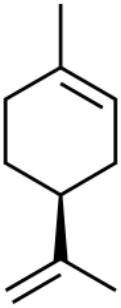
Figure 1.4 Skeletal structures of some saponins found in plants



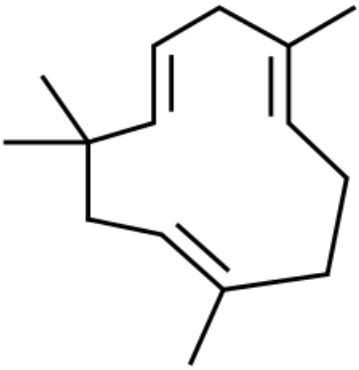
Thymol



Myrcene



Limonene



Humulene

Figure 1.5 Skeletal structures of some terpenes found in plan

1.6.3 Phytochemical Screening

Phytochemical research of a plant includes several aspects: (Mendoza and Silva, 2018)

- Extraction of the compounds to be analyzed from a sample or specimen.
- Separation and isolation of them.
- Identification and/or characterization of the isolated compounds.
- Investigation of the biosynthetic routes of a certain molecule.
- Determination or quantitative assessment

1.6.4 Role of Phytochemicals in Disease Prevention and Treatment.

Phytochemicals, the bioactive compounds found abundantly in plants, encompass a diverse array of substances, including alkaloids, tannins, saponins, flavonoids, phenols, steroids, and carotenoids. These compounds serve as the cornerstone of plant health and vitality, contributing not only to their growth and development but also to their defense mechanisms against environmental stressors and pathogens (Asaduzzaman & Asao, 2018).

In recent years, there has been a growing body of research highlighting the multifaceted health benefits of phytochemicals in humans. Studies have demonstrated their preventive properties in a range of health conditions, including inflammation, diabetes, aging-related disorders, infectious diseases, mental health disorders, cancer, oxidative stress, and wound healing (Liu, 2013; Pandey *et al.*, 2016).

For instance, flavonoids, a class of phytochemicals abundant in fruits and vegetables, have been extensively studied for their anti-inflammatory and antioxidant effects. Quercetin, one of the most widely studied flavonoids, has shown promise in reducing inflammation and oxidative stress, thereby mitigating the risk of chronic diseases such as cardiovascular diseases and cancer

(Panche *et al.*, 2016). Similarly, saponins, another group of phytochemicals found in various plant sources, have been recognized for their antimicrobial and immunomodulatory properties. These compounds have been investigated for their potential role in boosting the immune system and protecting against infectious diseases (Sedighi *et al.,* 2019).

The carotenoids, known for their vibrant colors in fruits and vegetables, have been associated with numerous health benefits, including vision protection, antioxidant activity, and reducing the risk of certain cancers and chronic diseases (Johnson, 2002). As our understanding of phytochemicals deepens, there is increasing interest in incorporating phytochemical-rich foods into dietary patterns to optimize health and well-being. By harnessing the power of nature's pharmacy, individuals can leverage the pharmacological properties of phytochemicals to support overall health and disease prevention.

1.7 Subchronic Toxicity Studies

Subchronic toxicity screening studies are conducted to evaluate the potential adverse effects of a substance when administered repeatedly over a relatively short duration, typically ranging from a few weeks to several months. This assessment involves examining the toxicological impacts of the substance following repeated exposure over a timeframe that covers a significant portion of an organism's lifespan but falls short of its entire lifespan. Subchronic toxicity screening is commonly carried out within a period of 28 to 90 days. These investigations play a critical role in assessing the safety profiles of various substances, including pharmaceuticals, chemicals, food additives, pesticides, and other compounds intended for human or animal use. (Gad,2014)

This screening process involves subjecting test subjects to repeated doses of the substance under investigation, mimicking real-world exposure scenarios. By monitoring various parameters such

as clinical signs, body weight changes, hematological and biochemical parameters, histopathological alterations, and organ function, researchers can identify any potential toxic effects that may arise during the subchronic exposure period (European Medicines Agency, 2009).

Subchronic toxicity studies are designed to bridge the gap between acute toxicity assessments, which focus on immediate adverse effects following a single exposure, and chronic toxicity evaluations, which explore long-term effects over an extended period. By conducting subchronic toxicity screening, researchers can gather crucial data on the substance's safety profile, informing regulatory decision-making and risk assessment processes (Organization for Economic Co-operation and Development, 2008).

1.7.1 Regulatory Requirements

The regulatory requirements and study design for subchronic toxicity screening studies typically include the following key aspects:

- Nonclinical laboratory studies must be conducted according to U.S. FDA GLP regulations (FDA's Redbook, 2003)
- Study Design Considerations: The study design should include considerations for the test animals, test substance, experimental design, observations and clinical tests, necropsy, and microscopic examination

1.7.2 Study Design

The design of a subchronic toxicity study is critical for obtaining reliable and meaningful data. Important considerations include:

1. **Test Substance Selection:** The test substance (e.g. drug candidate, chemical) is selected based on its intended use and potential for human or environmental exposure. The test substance used in toxicity studies should be the same substance that the petitioner intends to market. (FDA's Redbook2000, 2003). It is important that Proper characterization and dosing of the test substance is done before the toxicity study begins.
2. **Dose Selection**: In toxicity studies, selecting appropriate doses is crucial and should be guided by the known toxicity profile of the test substance. It is generally recommended to include at least three dose levels of the test substance, alongside a control group, to ensure comprehensive evaluation. When designing these studies, several factors must be considered:

   1. The highest dose should be adequate to elicit toxic responses in test animals, providing clear evidence of the substance's potential adverse effects.

   2. The lowest dose should not induce any toxic responses in test animals, ensuring that it serves as a baseline for comparison and does not confound the interpretation of results.

   3. The intermediate dose should be sufficient to trigger minimal toxic effects, such as subtle changes in enzyme levels or body weight, allowing for dose-response assessments without causing severe harm.

It is imperative that none of the administered doses result in fatalities, as this could compromise the integrity of the data collected. Additionally, all dose groups should receive the test substance concurrently to maintain consistency and facilitate accurate comparisons.

In terms of controls, including a concurrent control group is essential to establish a baseline for comparison against the test groups.

3. **Duration**: The study duration should be sufficient to detect delayed or cumulative toxic effects. Typically, subchronic studies last for 28, 90, or 180 days, depending on the regulatory requirements and the nature of the substance being tested.

4. **Animal Model**: The choice of animal species is important and should be based on factors such as the relevance of the model to humans, availability, sensitivity to the test substance and cost. Commonly used species include rats, mice, dogs, and non-human primates. This is done to ensure statistically significant results.

   Test animals must be described based on their species, strain (including substrain), sex, age, and weight. Additionally, each animal should be given a form of identification

5. **Route of Administration:** The route of administration should mimic human exposure as closely as possible. Common routes include oral, inhalation, dermal, and intravenous.

6. **Endpoints:** The study should include a comprehensive set of endpoints to assess systemic toxicity, target organ toxicity, and any other relevant effects. These endpoints may include clinical observations, body weight changes, food consumption, hematology, clinical chemistry, and histopathology.

7. **Statistical Analysis:** Statistical analysis should be performed to determine the significance of any observed effects. Sample size calculations should be based on the expected variability and the desired power of the study.

1.7.3. Importance of Subchronic Toxicity Screening in Herbal Medicine

Subchronic toxicity screening is crucial in ensuring the safety and efficacy of herbal medicines. By subjecting herbal products to rigorous testing over an extended period, researchers can uncover potential adverse effects that may arise from prolonged usage, thereby safeguarding the health of consumers (Obidike et al., 2013).

One of the primary reasons for conducting subchronic toxicity screening in herbal medicine is the presence of toxic secondary metabolites in many plants. These compounds, produced as natural defenses against adverse conditions, can pose risks to human health if consumed in large quantities or over extended periods (Anywar et al., 2020). Through systematic toxicity testing, researchers can identify and quantify these risks, allowing for informed decision-making regarding the safe use of herbal remedies.

Subchronic toxicity screening serves to identify and mitigate the unpredictable and complex effects that may arise from polyherbal preparations, commonly administered by skilled herbalists. While these preparations may offer therapeutic benefits, the interactions among individual components can result in unforeseen adverse reactions. By rigorously evaluating the safety of such formulations, researchers can mitigate potential harm and ensure the overall efficacy of herbal treatments (Obidike et al., 2013).

The main goal of toxicological evaluations for herbal medicines is to pinpoint any harmful effects and establish the threshold at which these effects manifest. Evaluating the safety of herbal drugs involves considering both the type and importance of any adverse effects, as well as the level of exposure at which they are observed. Conducting toxicity tests is crucial as it uncovers

potential risks linked to the usage of herbal remedies, particularly for vulnerable groups. Another critical purpose of these tests is to identify any harmful plant extracts or related compounds during the initial and final phases of drug development from plant-based sources. This helps in recognizing harmful substances that can be either eliminated or altered to enhance safety, thus allowing for a thorough assessment of more secure and promising alternatives. In some cases, making changes such as lowering the dosage or tweaking the chemical structure can make certain compounds more tolerable. (Obidike *et al.,*2013)

1.8 Histopathological Studies

Histopathology is the microscopic examination of tissue to study the manifestations of disease. It combines the study of tissue structure (histology) with the study of disease (pathology) (Mallick, 2024, National Cancer Institute, 2024)

By examining tissue samples under a microscope, pathologists can identify abnormalities in cell morphology, tissue architecture, and cellular organization, providing valuable insights into the underlying disease process.

1.8.1 Techniques in Histopathological Examination of Albino Rats

Histopathological examination of albino rats involves several techniques to prepare and analyze tissue samples for microscopic evaluation. Here are some key techniques used in histopathological examination:

1. Tissue Collection: Albino rats are euthanized, and tissue samples are collected from various organs of interest, such as the liver, kidneys, spleen, heart, lungs, gastrointestinal tract, and brain. (Bharathi *et al*.,2022)

2. Tissue Fixation: Tissue samples are immersed in a fixative solution (e.g., formalin) to preserve cellular structures and prevent degradation. Fixation helps to maintain the integrity of the tissues during subsequent processing steps.

Embedding: Dehydrated tissue samples are infiltrated with a liquid embedding medium, such as paraffin wax, which solidifies to form a block containing the tissue specimen. Embedding provides support for tissue sectioning and allows thin sections to be cut for microscopic examination.

5. Sectioning/Microtomy: The embedded tissue blocks are sectioned into thin slices (usually 4-6 micrometers thick) using a microtome. These thin sections are mounted onto glass slides for staining and microscopic analysis.

Staining: The tissue sections are stained to enhance contrast and visualize cellular structures. Hematoxylin and eosin (H&E) staining is commonly used to distinguish between nuclei (stained blue-purple) and cytoplasm (stained pink). Additional staining techniques may be employed to highlight specific structures or cell types.

Microscopic Examination: The stained tissue sections are examined under a light microscope by a trained histopathologist. Microscopic evaluation allows for the assessment of cellular morphology, tissue architecture, and the presence of pathological changes such as inflammation, necrosis, or tumor formation.

- Photomicrography: Images are captured for documentation and further analysis.

The correlation between biochemical constituents and tissue morphology is a fundamental aspect of biomedical research, particularly in the fields of histopathology and pharmacology.

The relationship between biochemical constituents and tissue morphology is intimate and complex. For example, the collagen content in connective tissues directly affects their strength

and elasticity. Variations in the biochemical composition can lead to changes in tissue morphology, which may impact the tissue's mechanical properties and functionality (Mienaltowski, 2021)

1.8.2 Histopathology Studies And Biochemical Parameters

Understanding the correlation between biochemical constituents and tissue morphology is paramount in various scientific endeavors, particularly in the field of tissue engineering. In tissue engineering, the primary objective is to develop biomaterials that closely mimic the natural biochemical and morphological characteristics of native tissue (Mercatelli, 2019). This entails a deep understanding of how the biochemical composition of tissues influences their overall morphology and function.

Aberrations in tissue morphology, such as the presence of tumor cells or inflammatory infiltrates, often coincide with distinct biochemical changes within the affected tissue (Jones & Smith, 2020). These biochemical changes may include alterations in protein expression patterns, metabolic pathways, or the accumulation of specific biomolecules.

An intriguing aspect of this correlation is its implications for disease diagnosis and prognosis. By identifying and analyzing specific biochemical constituents within tissues, clinicians can effectively use them as diagnostic biomarkers for various diseases (Brown & Johnson, 2019). For instance, immunohistochemical detection of specific protein markers can aid in distinguishing between different types of cancer or assessing the severity of a disease (Garcia & Perez, 2020). This integration of biochemical analysis with tissue morphology assessment enhances diagnostic accuracy and enables personalized treatment approaches tailored to individual patients.

Understanding the relationship between biochemical constituents and tissue morphology offers information regarding the physiological and pathological processes at the molecular level. By understanding how changes in biochemical composition influence tissue morphology, researchers can unravel the underlying mechanisms driving disease progression and identify potential therapeutic targets (Wang & Liu, 2017). This approach, bridging the fields of molecular biology and pathology, is instrumental in advancing our understanding of complex diseases and developing innovative therapeutic strategies.

1.9 Justification of the study

The exploration of *Picralima nitida* roots through phytochemical screening and subchronic toxicity studies is justified by the need to understand the toxic nature of the plant being used by people. Despite the widespread use of *Picralima nitida i*n traditional medicine, there is hardly any experimental data regarding its safety and toxicity, especially concerning its long-term effects on health. The study aims to put herbal medicines into contemporary healthcare systems and ensure the well-being of individuals who rely on such remedies.

1.10 Aims and Objectives

Aim:

This project aims to investigate the phytochemical composition and evaluate the subchronic toxicity of *Picralima nitida,*to assess its safety profile for human consumption or therapeutic use.

Objectives:

-To determine the phytochemical constituents in the roots of *Picralima nitida*

- To evaluate the potential adverse effects of *Picralima nitida roots* on systemic health and organ function of rats over an extended period of exposure.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Materials

2.1.1 Solvents and Reagents

Methanol (99.8%), Distilled water, Ethanol, chloroform, Normal saline, Formalin saline.

Dragendorff's reagent, Fehling's solution, Acetic anhydride, Molisch's reagent, Benedict's reagent, Ninhydrin reagent, Liebermann-Burchard reagent, Hydrochloric acid (HCl), Sulfuric acid (H_2SO_4), Sodium hydroxide (NaOH), Potassium hydroxide (KOH), Acetic acid, Ferric Chloride, 1% Gelatin solution, Sodium chloride (NaCl), Petroleum ether, Ammonia solution (NH_4OH)

The above chemicals and reagents used to conduct the research work were all of analytical grade.

2.1.2 Glassware

Measuring cylinder (2000ml), conical flask, beakers (250ml, 500ml), round bottom flask, test tube, test tube rack, tongs, spatula, porcelain dish, glass jars (big, medium, small), Glass stirrer, funnel, bottles, pipette (5ml, 10ml),

2.1.3. Equipment

Condenser, freezer, weighing balance, weighing scale, rotary evaporator, Centrifuge

2.1.4 Other Materials

Cotton wool, Filter papers,Syringes (1 mL and 5 mL), surgical blades, scapels and scissors, and surgical hand gloves, Microscope Slides, Cardboard, Aluminium Foil paper.

2.2 Methods

2.2.1 Plant collection and Identification.

The roots of the plant were obtained from a local market (New Benin Market under Oredo Local Government Area, Benin City) in February, 2024. The plant roots were identified in the herbarium unit of the department of plant biology and biotechnology where the voucher number FHI109429 was given.

2.2.2 Extraction of crude sample.

The roots of the plant were cut into pieces and oven dried for about 24 hours until it was fully dried. The dry roots were pulverized with a mechanical grinder and then weighed using a weighing scale. It weighed 0.7Kg.

0.6Kg of the ground plant root was extracted by maceration in 1.5L of methanol (99.8% purity) for 72 hours. This was followed by filtration with a funnel and Whatman No 1 filter paper. The filtrate was concentrated using a rotary evaporator at 45°C to obtain the crude methanolic root extract of *Picralima nitida.*

The extract was stored in an air-tight container and kept in the refrigerator at 4°C until further use.

2.2.3 Animals

The animals (99 – 160 g) used were male Wister rats, obtained from the Animal House, Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin). They were acclimated at an ambient temperature of 27- 30°C for one week.

They were fed with standard rodent pellets (Bendel feeds and Flour Mill Ltd., Ewu, Nigeria) and given water *ad libitum.* The animals were exposed to lighting conditions and handled according to protocol approved by the Faculty of Pharmacy ethical committee Ethical approval was obtained from the Animal Ethics Committee, Faculty of Pharmacy University of Benin, Benin City, Nigeria before the study was carried out.

2.2.4 Phytochemical Screening of the powdered roots of *Picralima nitida*

Standard methods were used to determine the presence of phytochemicals such as: carbohydrates, saponins, alkaloids, anthraquinones, tannins and other phenolics by simple chemical tests (Sofowora, 1983)

The crude powdered sample (5 g) was boiled in 75 mL of distilled water for 30 minutes. The solution was filtered while still hot using a filter paper and allowed to cool. The filtrate obtained was used to carry out the following tests.

2.2.4.1 General Tests for Alkaloids

Dragendorff's reagent (2 drops) was added to 2 mL of the filtrate.

Expected observation for a positive result: formation of a reddish brown precipitate.

2.2.4.2 Tests for Carbohydrates

Molisch's Test

Two drops of 1% alcoholic naphthol followed by 2 mL of concentrated sulphuric acid were added to 2 mL of the filtrate in a slanted position.

Expected observation for a positive result: formation of a violet ring at the interface of two liquid layers.

2.2.4.3 Tests for Reducing Sugars

Fehling's test: 2 drops of Benedict's reagent (a mixture of equal volumes of Fehling's solution A and B) was added to 2 mL of the filtrate. The resulting solution was heated over a boiling water bath for 3 minutes.

Expected observation for a positive result: formation of orange or brick red precipitate.

2.2.4.4 Keller Kiliani's test for deoxysugars

Few drops of dilute acetic acid containing a trace of 5% ferric chloride was added to 2 mL of filtrate. The resulting mixture was transferred to the surface of concentrated sulphuric acid.

Expected observation for a positive result: formation of a violet ring at the interface of two liquid layers.

2.2.4.5 Test for saponins

Frothing Test

Distilled water (10 mL) was used to dilute 1 mL of the filtrate and shaken vigorously for one minute.

Expected observation for a positive result: formation of a persistent frothing.

2.2.4.6 Test for Tannins

Gelatin test: To 2 mL of the aqueous filtrate was added 2 mL of 1% gelatin solution in 10% NaCI. Expected observation for positive result: formation of precipitate.

2.2.4.7 Test for phenolic compounds

Ferric chloride test

Distilled water (5 mL) was added to 2 mL of filtrate then 2 drops of 5% ferric chloride solution was also added. A blank test was carried out by adding 2 drops of 5% ferric chloride solution to 5 ml of distilled water.

Expected observation for a positive result: formation of intense coloration in the test sample.

2.2.4.8 Test for Flavonoids

Lead acetate test.

Few drops of lead acetate solution were added to 2 mL of filtrate. Expected observation for a positive result: formation of milky precipitate.

2.2.4.9 Test For Anthraquinone Derivatives

Bontreger's test: Petroleum ether (2 mL) was shaken with 2 mL of filtrate. The ether layer was then washed with 2 mL distilled water and then shaken with dilute ammonia solution.

Expected observation for a positive result: Formation of pink colour on addition of ammonia solution.

2.2.4.10 Test For Proteins

Xanthoprotenic test

Few drops of concentrated Nitric acid were added to 2 ml of filtrate.

Expected observation for positive result: formation of a yellow precipitate.

2.2.5 Subchronic toxicity

2.2.5.1 Dose calculation

The plant extract was dissolved in water and a stock solution of 50mg/mL was made by weighing 5g of the plant extract in 100ml of distilled water.

The volume given to the rat was gotten using the formula:

$$\text{Volume of drug to be given (ml)} = \frac{weight(kg) \times dose(\frac{mg}{kg})}{stock\ solution\ (\frac{mg}{ml})}$$

2.2.5.2 Animal Groupings and Weighings

The twenty-four animals were randomly divided into four groups each containing six (6) animals. The control animals (Group 1) had no treatment. Group 2, Group 3 and Group 4 were given the plant extract at 50mg/Kg, 100mg/Kg and 200mg/Kg, respectively, once daily for 28 days using an orogastric tube.

On the 29th day, the rats were weighed before they were sacrificed. Chloroform was used as an anesthetic to euthanize the animals.

2.2.5.3 Biochemical analysis

Blood was taken from each rat using a 5ml syringe into a plain sample bottle and allowed to stand on the laboratory bench in an inclined position for 15 minutes and then centrifuged at 3000 rpm for 10 minutes. The resulting serum was transferred into an appropriately labeled bottle for serum biochemical evaluations. The bottles were sent to the chemical pathology unit of University of Benin Teaching Hospital (UBTH) for the biochemical evaluations.

2.2.5.4 Histopathology of Animal Organs

After euthanasia, a midline incision was made on the ventral aspect starting from the base of the neck down to the umbilical. The liver, heart and kidneys were carefully cut off, washed with normal saline solution and put in Universal bottles containing formalin.

The tissues were fixed in normal formal saline for 72 hours. These tissues were completely dehydrated in ascending concentration of alcohol (70, 90, 96 and 100%). They were further treated with paraffin wax. They were allowed to solidify before sectioning took place.

Sections of the tissues were then cut on a microtome to 4 micrometer. These were then fixed on a slide and allowed to dry. The samples were subsequently stained in hematoxylin-eosin and examined under a microscope at x400 magnification. Photomicrographs of the samples were taken and recorded.

2.2.5.5 Statistical analysis

Data was analyzed using Google sheets. The probability of $*p<0.05$ was considered to be statistically significant. All data were represented as mean ± standard error of mean. The graphs was generated by Google sheets.

CHAPTER THREE

RESULTS

3.1. Organoleptic Properties

Colour- Brown
Taste: Bitter
Texture: Rough

3.2 Phytochemical Screening

Phytochemical screening of the plant extract is presented in the table below.

Table 3.1- Showing the result of phytochemical tests done on *Picralima nitida*

Phytochemicals	**Inference**
Alkaloids	+
Anthraquinones	-
Carbohydrates	+
Deoxy sugars	+
Flavonoids	+
Phenolic	+
Proteins	-
Reducing Sugars	+
Saponins	+
Steroidal Saponins	-
Tannins	-

Key: +Positive; -Negative

3.3 Effect of plant extract on Kidney function parameters

The renal biochemical markers are presented in the table below.

Table 3.2 Effect of roots of *Picralima nitida* on Kidney function parameters

Groups	Urea(mmol/L)	Na+mmol/L	K+ (mmol/L)	HCO3- (mmol/L)	Cl- (mmol/L)	Creatinine(mg/dl)
Control	44 ±1.37	139.75±1.71	4.1 ±0.06	21.75± 0.98	103.5±1.47	0.475 ±0.03
50mg/Kg	42 ± 2.01	139.40±1.49	4.0±0.12	26.2 ±2.30	102 ±1.02	0.42 ±0.02
100mg/Kg	40.33 ± 1.63	140.67±1.58	4.0±0.15	21.167±0.82	101.17±1.75	0.417 ±0.03
200mg/Kg	36.33 ± 1.19	140.67±1.15	3.9 ±0.14	19.83 ±0.26	102.33±0.94	0.45 ±0.02

Results were shown with the standard error from the mean.

3.4 Effect of plant extract on Lipid profile parameters

Table 3.3 Effect of roots of *Picralima* nitida on Lipid profile parameters

Groups	TC(mg/dL)	TG(g/dL)	HDL(mg/dL)	LDL(mg/dL)
Control	95.75 ±5.67	99.75 ±11.68	31.75 ±7.21	49 ±7.78
50mg/Kg	107.4 ±5.67	96.8 ±9.18	36.8 ±5.94	51.4 ±5.02
100mg/Kg	89.67 ±5.92	119.17 ±14.33	36.5 ±3.46	34±6.12
200mg/Kg	110.67 ±5.65	96.83 ±9.18	44 ±5.75	47.17 ±2.93

TC- Total Cholesterol

TG- Triglycerides

HDL- High Density Lipoprotein

LDL- Low Density Lipoprotein

3.5 Effect of Plant extract on Liver function Parameters

	AST(U/I)	ALT(U/I)	ALP(U/I)	TB(mg/dL)	DB(mg/dL)	TP(g/dL)	ALB(g/dL)	GLO(g/dL)
CONTROL	138±7.4	75.25 ±4.72	391.5 ±31.80	0.225 ±0.018	0.1	7.45 ±0.199	3.2±0.061	4.00±0.086
50mg/Kg	151.6 ±8.88	68.20 ±3.15	372.8 ±11.91	0.24 ± 0.022	0.1	7.22 ±0.23	3.16± 0.05	4.14 ±0.23
100mg/Kg	146.5± 19.43	69.26 ±5.25	474.5 ±75.21	0.25 ±0.02	0.1	7.2 ±0.196	3.16 ±0.05	4.03± 0.178
200mg/Kg	119 ±11.20	54.26 ±3.01	301.83 ±23.56	0.22 ±0.0093	0.1	7.47 ±0.12	3.2± 0.068	4.27±0.177

Table 3.4 Effect of *Picralima nitida* roots on Liver function parameters

AST -Aspartate Aminotransferase, ALT -Alanine Aminotransferase, ALP -Alkaline Phosphatase

TB -Total Bilirubin, DB -Direct Bilirubin, TP- Total Protein, ALB- Albumin, GLO -Globulin

3.6 Effect of Plant Extract On Body Weight Of Rats

The weights of the rats were compared on the first day of the experiment and on the day they were to be sacrificed.

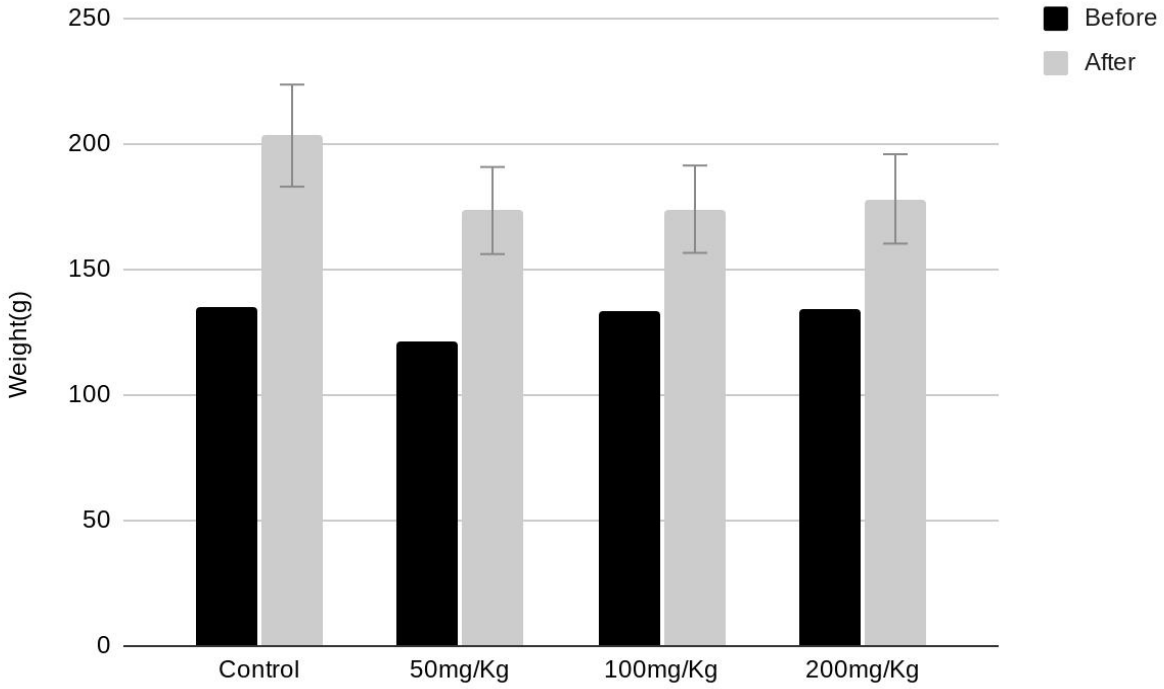


Figure 3.1 Effect of Plant extract on body weights of rats

3.7 Effects of Plant extract on organ weights of rats.

The weights of the organs are plotted on the graph below and compared with other groups

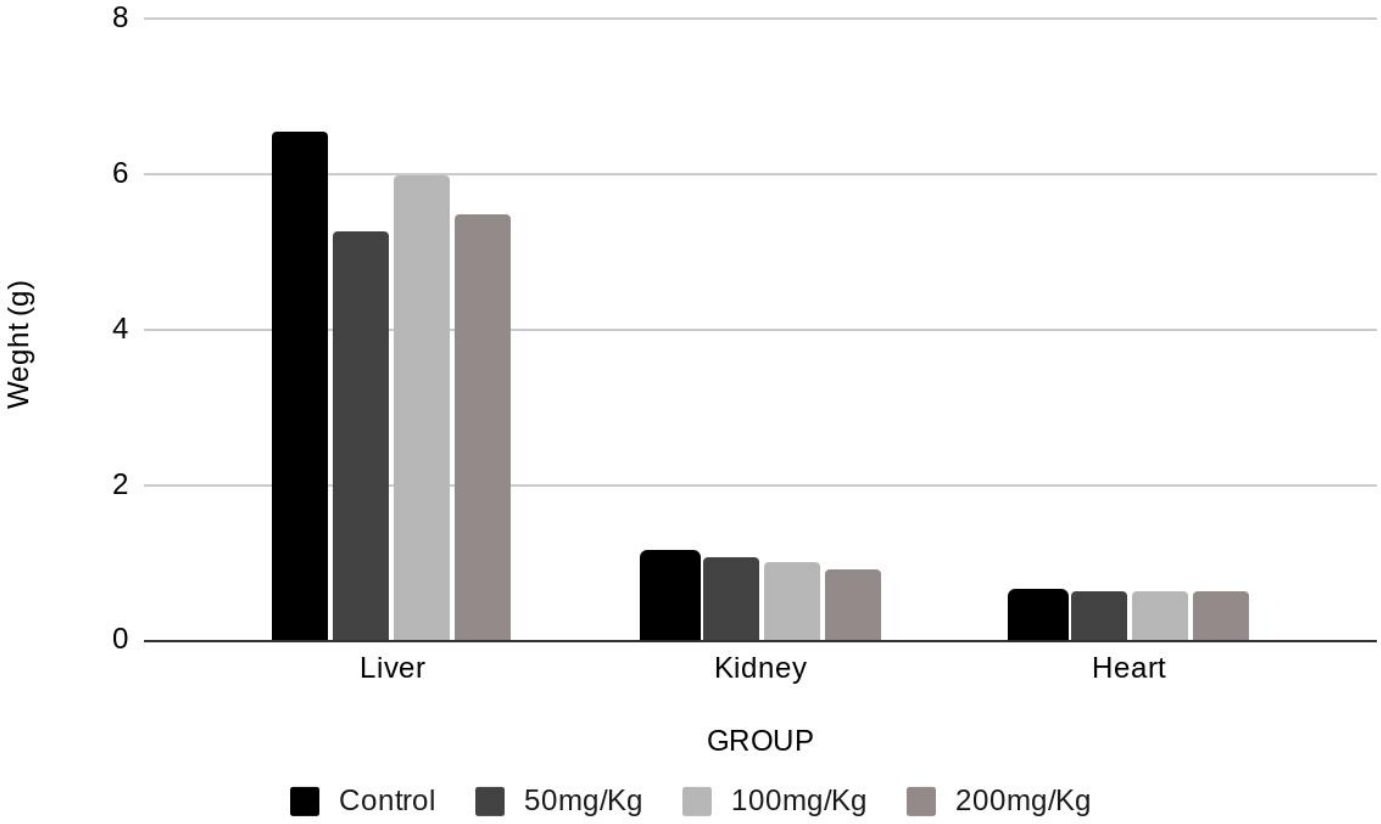


Figure 3.2: Effect of plant extract on organ weights of rats.

3.8 Histopathology Results

The images below were gotten from studying the organs (Liver, kidney and heart) gotten from the animals

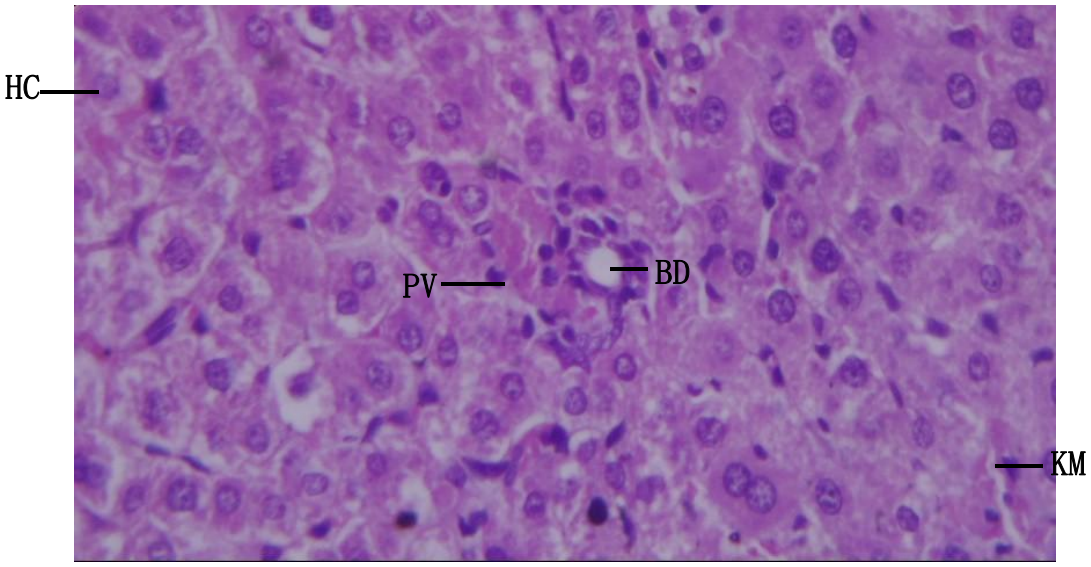


Plate 1. Rat liver. Control 2 showing: normal hepatocytes (HC), portal vein (PV) and bile duct (BD), Kupffer cell mobilization (KM): H&E x 400

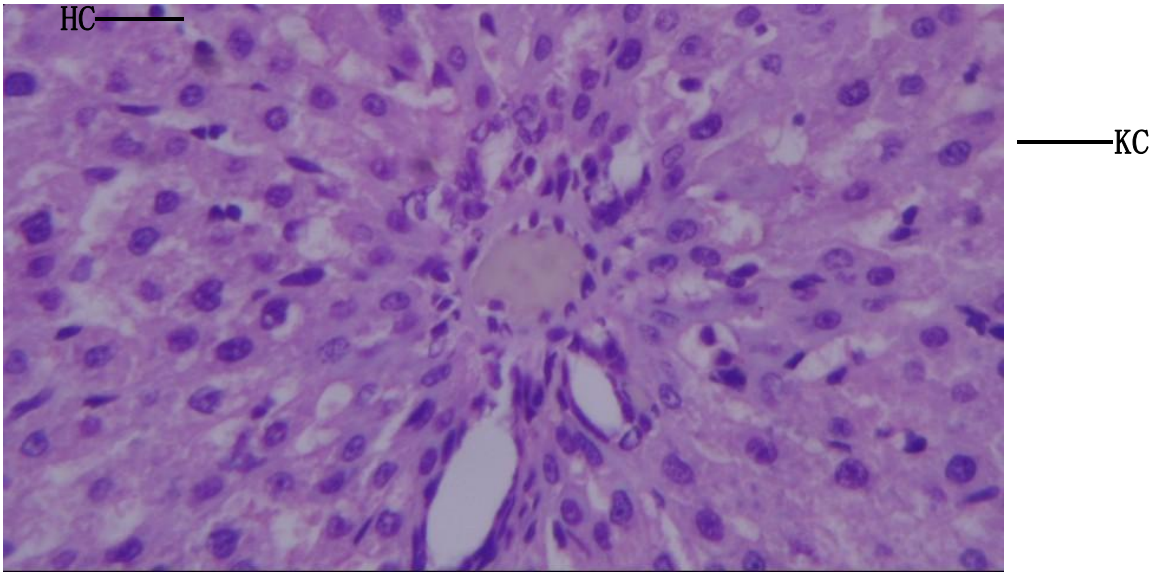


Plate2. Rat liver given 50mg P. initida root extract showing: normal

hepatocytes (HC), recruitment of sinusoidal Kupffer cells (KC): H&E x 400

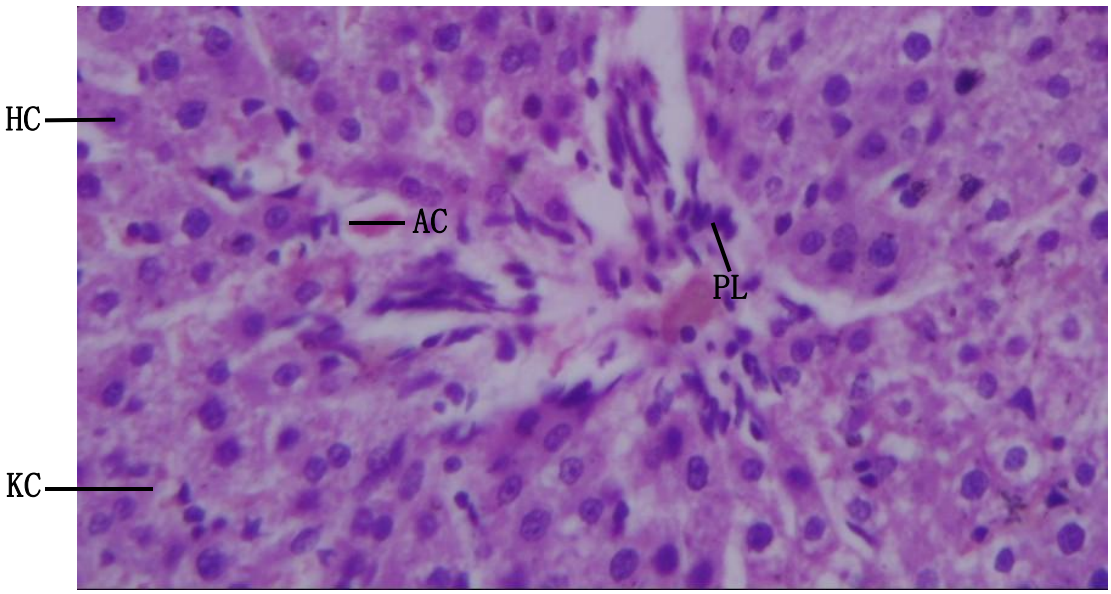


Plate 3. Rat liver given 100mg P. nitida root extract showing: normal hepatocytes (HC), recruitment of sinusoidal Kupffer cells (KC), active vascular congestion (AC) and mild periportal mobilization of lymphocytes (PL): H&E x 400

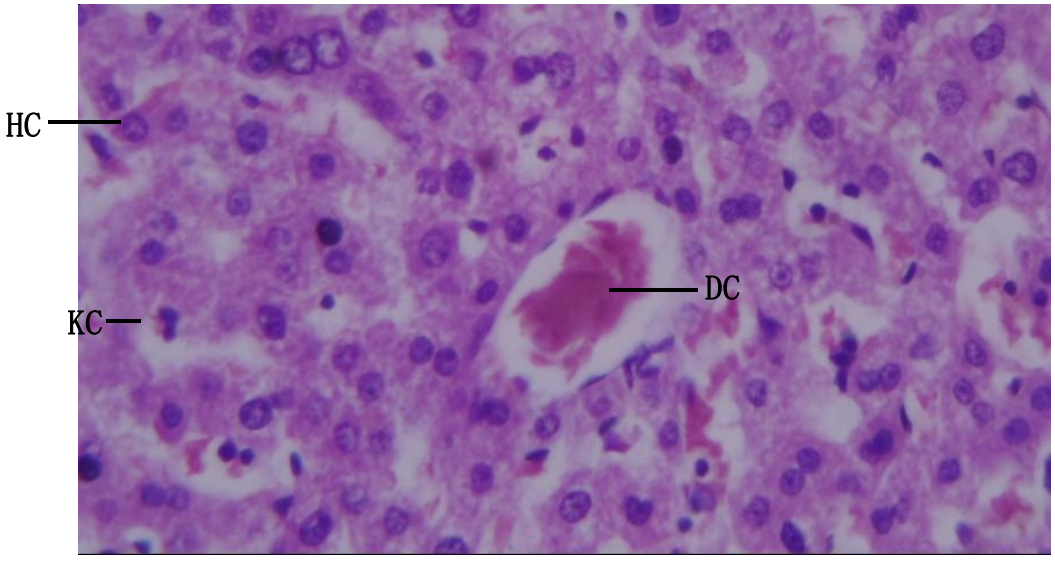


Plate 4. Rat liver given 200mg P. nitida root extract showing: normal hepatocytes (HC), active vascular congestion and vasodilatation (DC),

recruitment of sinusoidal Kupffer cells (KC): H&E x 400

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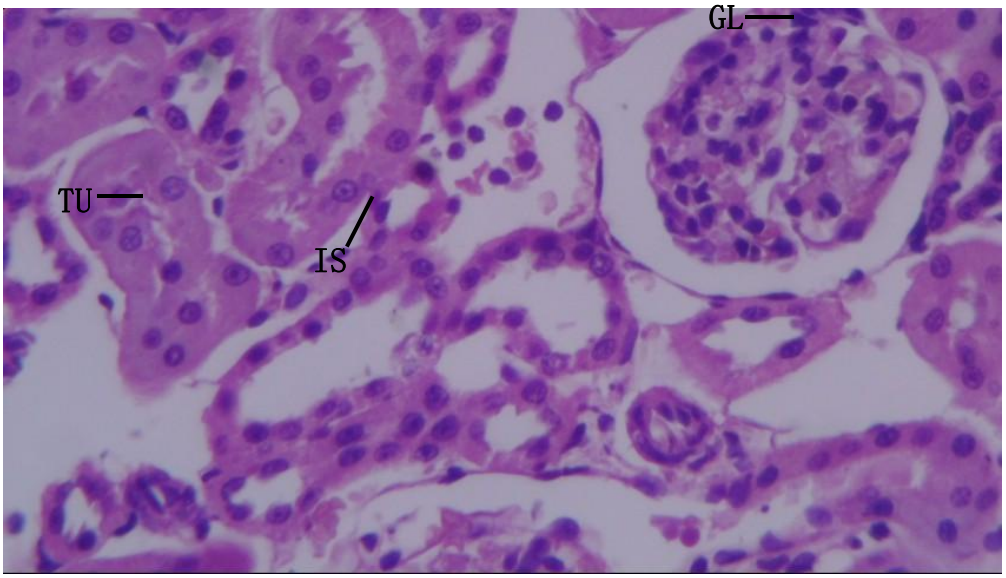

Plate 5. Rat kidneys. Control. Showing normal architecture: composed of: tubules (TU), glomerulus (GL) and interstitial space (IS): H&E x 400

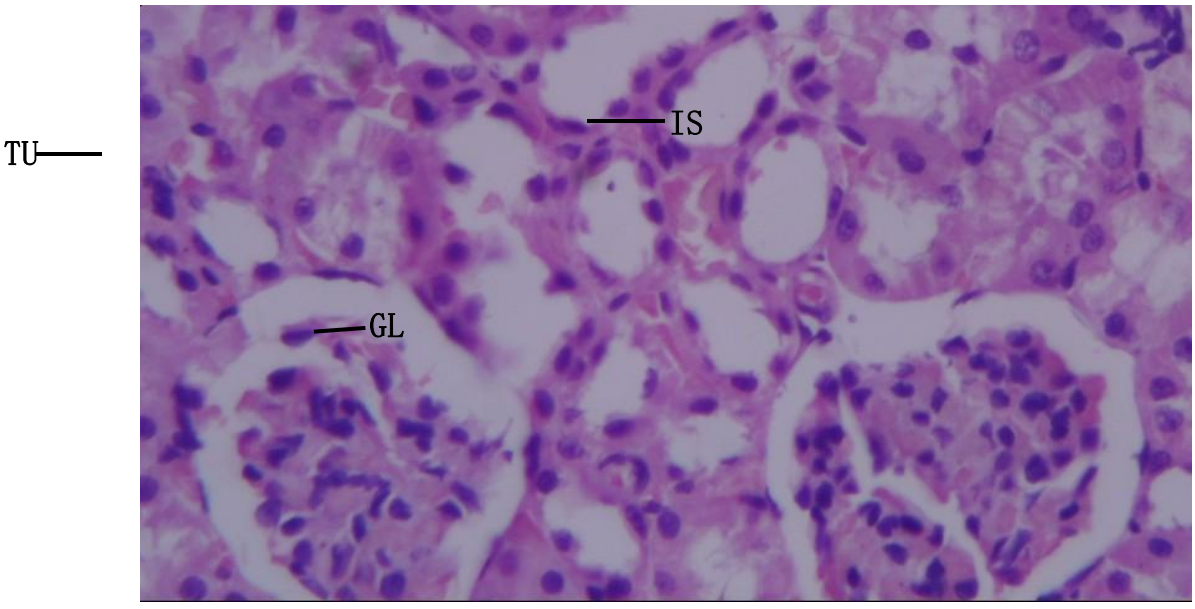

Plate 6. Rat kidneys. Control. Showing normal architecture: composed of: tubules (TU), interstitial space (IS) and glomeruli (GL): H&E x 400

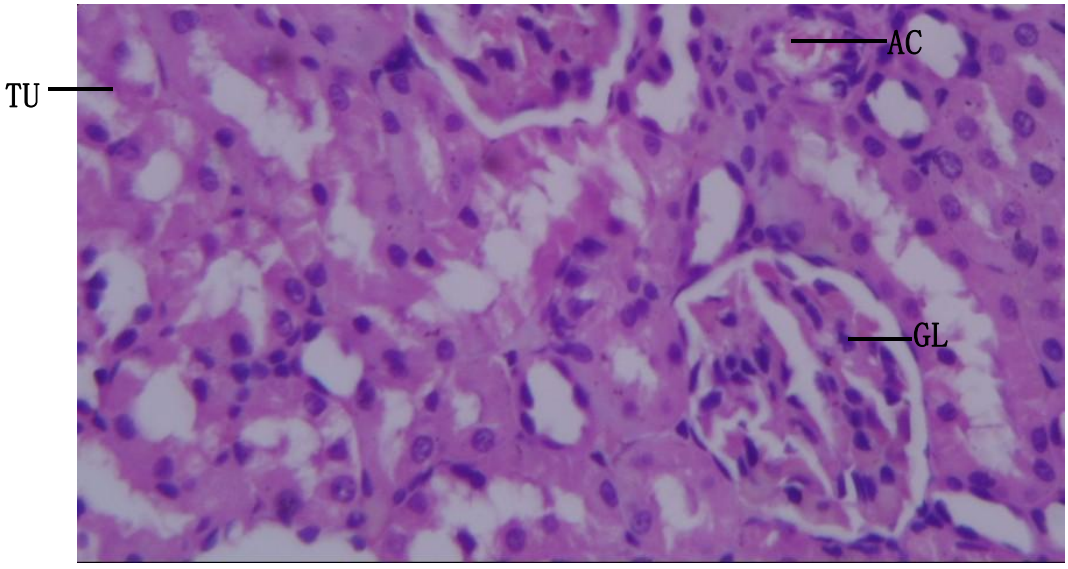

Plate 7. Rat kidneys given 50mg P.nitida- root extract showing: tubules (TU), active interstitial congestion (AC) and glomeruli (GL), all normal: H&E x 400

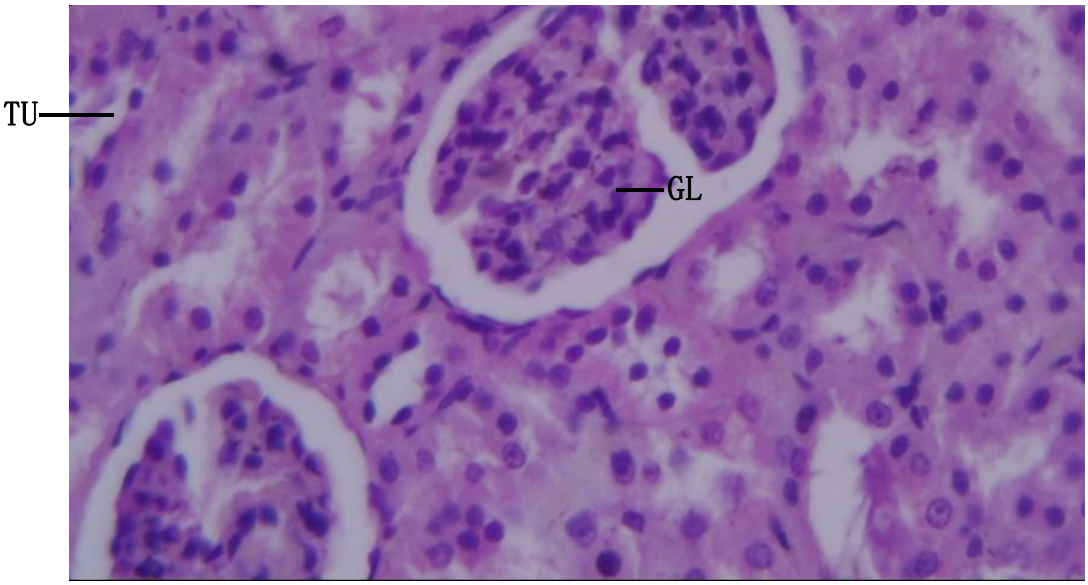

Plate 8. Rat kidneys given 100mg P. nitida root extract showing: normal

tubules (TU) and glomeruli (GL): H&E x 400

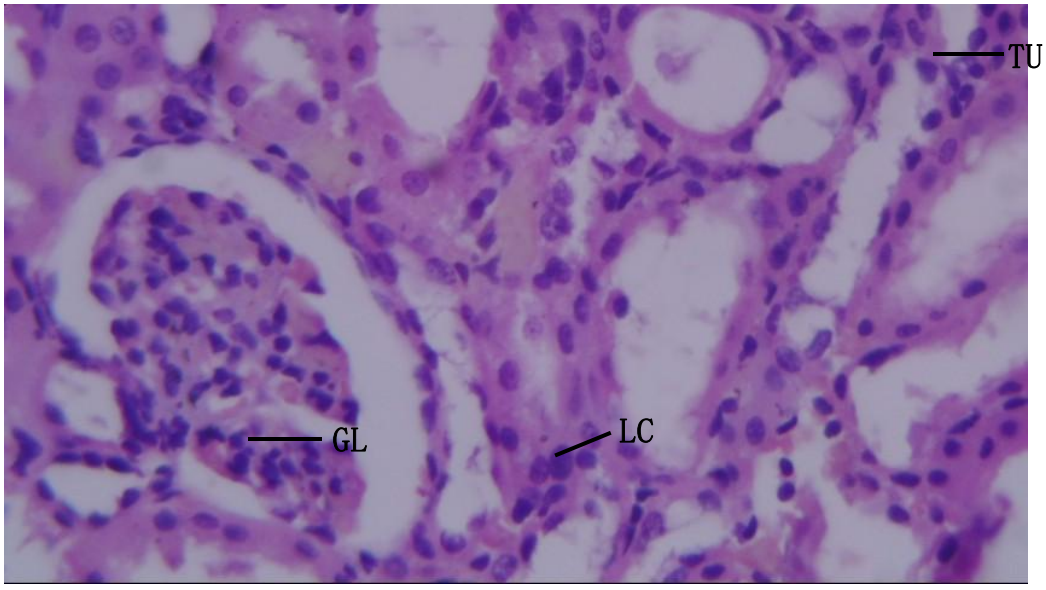

Plate 9. Rat kidneys given 200mg P. initida root extract showing normal architecture: tubules (TU), glomerulus (GL) and interstitial mobilization of lymphocytes (LC): H&E x 400:

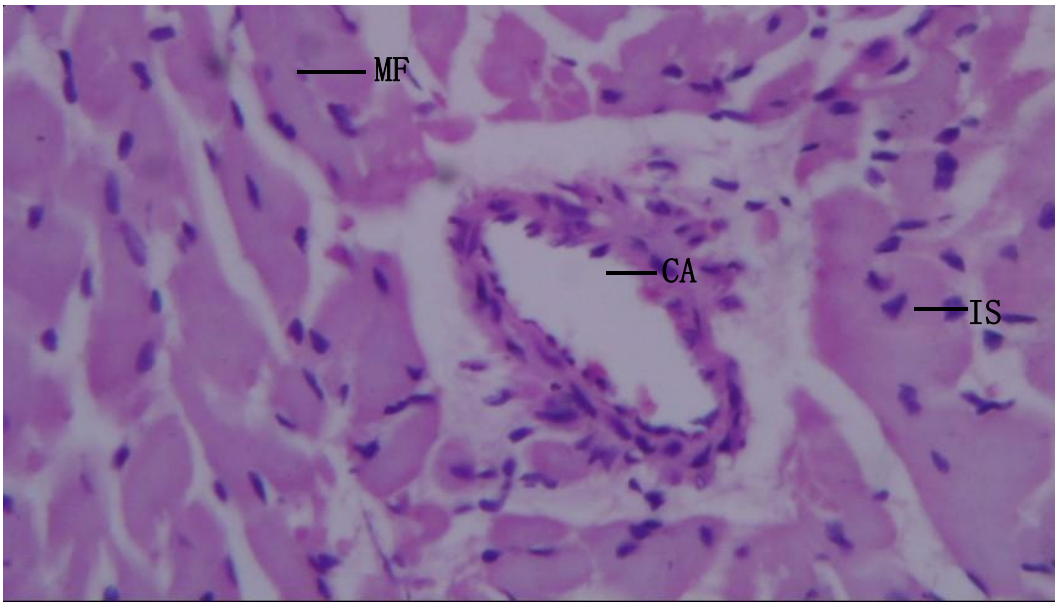

Plate 10. Rat heart. Control: showing normal architecture: bundles of myocardial fibres (MF), interstitial space (IS) and coronary artery (CA): H&E x 400

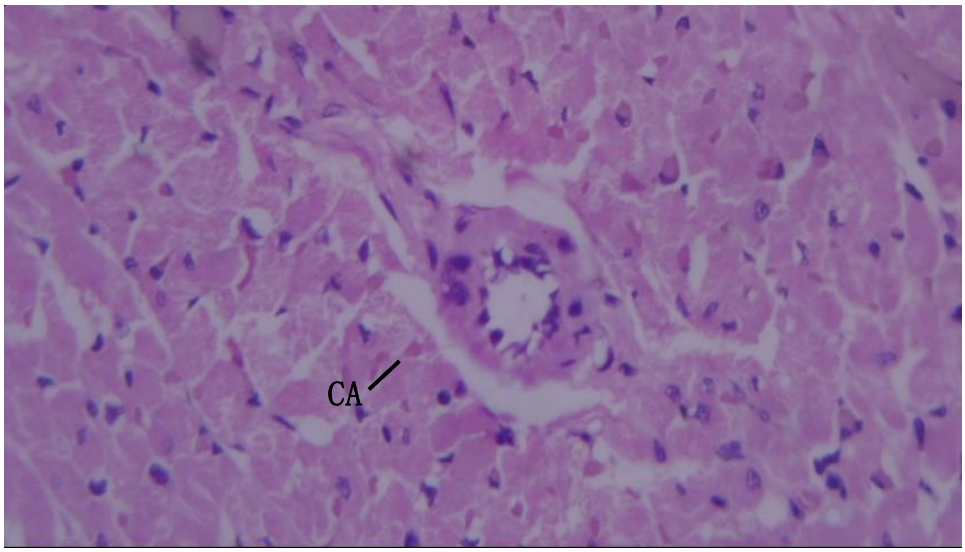

Plate 11. Rat heart given 50mg P. initida root extract showing: normal bundles of cardiomyocytes (CM) and coronary artery (CA): H&E x 400

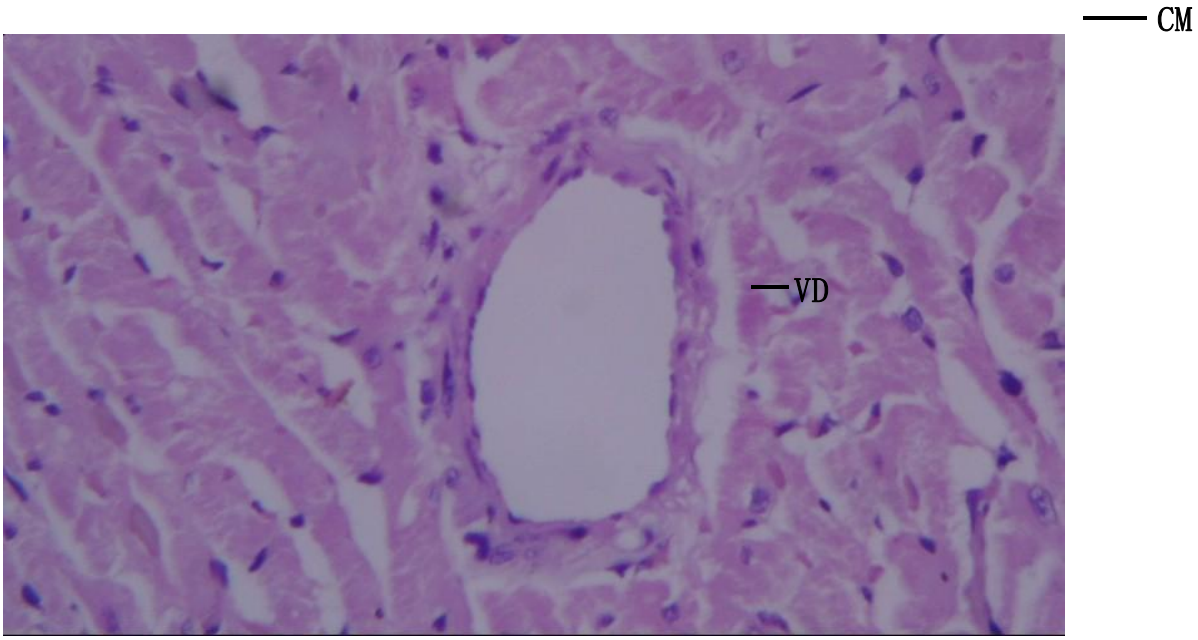

Plate 12. Rat heart given 100mg P. nitida root extract showing: normal bundles of cardiomyocytes (CM) and coronary vasodilatation (VD): H&E x 400

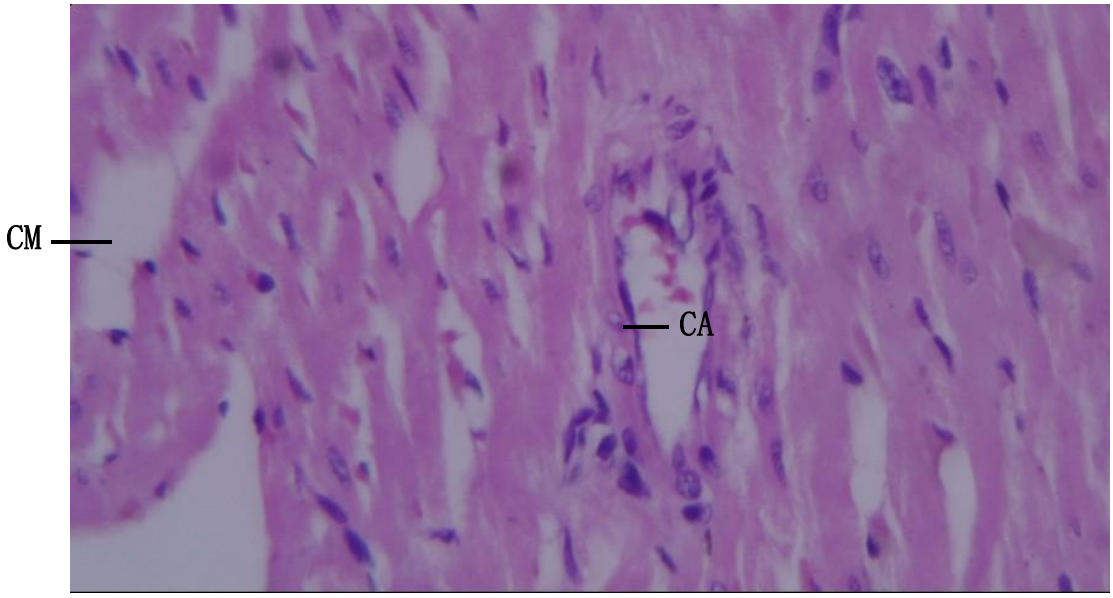

Plate 13. Rat heart given 200mg P. nitida root extract showing: normal architecture: bundles of cardiomyocytes (CM) and coronary artery (CA): H&E x 400

CHAPTER FOUR

DISCUSSION

4.1 Phytochemical screening in *Picralima nitida*

The phytochemical tests done on the plant extract showed the presence of alkaloids, carbohydrates, flavonoids, phenol, reducing sugars, saponins (check table 3.1). This is in line with preliminary phytochemical tests conducted on various crude extracts of P. *nitida* that have revealed the presence of some chemical groups: alkaloids, flavonoids, tannins, saponins, triterpenes and sterols. (NgaÃ¯ssona,2016).

Alkaloids, in particular, represent a major class of phytochemicals isolated from *P. nitida.*

Picralima nitida is known for its abundance of alkaloids, some of which exhibit notable biological activities (Corbett *et al.,* 1996; Fakeye *et al.,* 2000; Ramirez *et al.,* 2003).

The primary alkaloids extracted from *P. nitida* are indole alkaloids, including akuammine, pseudoakuammine, akuammidine, akuammicine, akuammigine, pseudoakuammigine, akuammiline, and akuammenine, named after the indigenous term for the plant in Ghana, "Akuamma" (Henry and Sharp, 1972).

Additional alkaloids have been identified and isolated from various parts of the plant. These include picraphylline, picracine, picraline, picralicine, picratidine, picranitine, burnamine, pericalline, and pericine (Ezeamuzine *et al.,*1994, Corbett *et al.,* 1996, Fakeye *et al.,*2000)

Although most of these alkaloids are predominantly found in the seeds of the plant, they can also be detected in other plant parts such as the leaves, bark, and roots.

These phytochemicals are known to be responsible for the various pharmacological effects that the plant exhibits.These alkaloids contribute to the pharmacological effects of *Picralima* nitida, such as antipyretic, anti-inflammatory, and analgesic effects observed in animal studies. (Kouassi

et al., 2015) It's important to note that the extraction and quantification methods can influence the specific alkaloids detected in plant samples.

For example, according to (Erharuyi et al., 2019) Akuammine, an indole alkaloid that is considered the main active component of the seeds has been found to have antimalarial properties.

4.2 Effect Of Plant Extract On Kidney Parameters

The kidneys are vital organs responsible for maintaining the body's internal balance by regulating fluid and electrolyte levels, filtering metabolic waste products from the blood, and excreting them through urine (Levey & Coresh, 2012).

Nephrotoxicity refers to kidney injury resulting from exposure to various substances, including drugs and environmental chemicals, either directly or indirectly (Sharma *et al.,* 2023). Drugs are capable of causing injury to the kidneys. The parameters used include serum creatinine, blood urea nitrogen (BUN), and electrolyte levels.

Elevated levels of serum creatinine may indicate impaired kidney function, as the kidneys are responsible for removing creatinine from the bloodstream. BUN levels may increase in conditions such as decreased renal function, dehydration, or increased protein breakdown. Electrolyte levels, including sodium, potassium, chloride, and bicarbonate, are closely regulated by the kidneys (Levey & Coresh, 2012).

Abnormalities in electrolyte levels may indicate kidney dysfunction or electrolyte imbalances secondary to toxicity or renal disease. The Kidney function of the animals was not affected as these biomarkers did not change significantly with treatments compared to control. This

observation is similar to Awodele *et al.,* (2019) and Lydia *et al*., (2015). In their study on the seeds and supported by no comparable difference between the weights of kidneys.

4.3 Effect of Plant Extract on Lipid Profile parameters

The result shows no significant difference in the Total Cholesterol, Triglyceride, LDL and HDL when compared to the control group. (Figure 3.2) This observation was witnessed in Otoo et al., 2015 and Awodele *et al.,* 2019. Therefore, there seems to be no risk of drug-dependent dyslipidemia, a well-established risk factor for cardiovascular diseases.

4.4 Effect of Plant Extract On Liver function Parameters

Hepatotoxic substances can cause damage to liver cells, leading to leakage of liver enzymes into the bloodstream. Elevated levels of liver enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) observed in Liver Function Tests may indicate hepatotoxicity.

Based on the results obtained, there was no significant difference between the amounts of ALT and AST obtained and compared with the control group. However, ALP was significantly elevated (p-value < 0.05) than those in the control group at the dose 100mg/Kg. There is no significant difference when compared to the control group with the other doses of the treatment. This was also observed in Awodele et al.,2019

This elevation could have resulted from the fact that ALP can be excreted from the bones and other tissues probably due to external causes. Therefore, elevated ALP levels can be indicative of bone disorders.

Cholestasis is a condition characterized by impaired bile flow from the liver, which can elevate ALP levels due to accumulation of bile acids in the liver and bloodstream. To determine if cholestasis is being observed at the 100 mg/Kg group, further tests need to be run.

4.5 Effect of Plant extract on Body and Organ weights

Body weight serves as a general indicator of the overall health and well-being of test animals. A decline in body weight may suggest systemic toxicity, metabolic disturbances, or impaired nutrient absorption, whereas stable or increasing body weight typically indicates normal physiological function. (Smith and Jones, 2018)

Substances with toxic effects can lead to changes in metabolic processes, organ function, or energy balance, resulting in alterations in body weight. A significant decrease in body weight may indicate systemic toxicity, organ damage, or physiological stress induced by the test substance.(Gupta & Mital, 2019)

From Figure 3.5. The weights of the animals increased significantly over the full course of the experiment. Although the control group showed a greater increase in weights, the treatment groups all had a significant increase.

4.6 Histopathological Results

The plates showing the organs of the control animals show normal hepatocytes, tubules and cardiac myoctes shown in Plates 1, 5 and 9 and showed no abnormal histology. In the liver cells, the treatment groups showed the recruitment of Kupffer cells for the various doses of the plant extract. This could mean that the liver cells were being triggered as Kupffer cells are known to be present in liver injury and hepatocellular necrosis.

The kidneys showed normal glomerulus and tubules in the control and treatment groups. However, at 200mg/kg, the interstitial spaces showed the presence of lymphocytes. Lymphocytes are rare in healthy kidneys.

The heart from the control group and treatment groups showed normal cardiomyocytes. The coronary artery was shown to be normal in all the groups. However, there was vasodilation in the different treatment groups. This vasodilation seems to increase in a dose dependent manner. Vasodilation is important in the treatment of cardio congestive problems and the plant can be explored for this use.

CHAPTER FIVE

CONCLUSION

In conclusion, the present study investigated the phytochemical constituents and biochemical effects of the methanol root extract of *Pricalinma nitida* on rats. Our findings revealed a diverse range of phytochemicals present in the extract, including alkaloids, flavonoids, tannins, saponins, and phenolic compounds, which are known for their potential health benefits.

Despite the phytochemical investigation revealing the presence of various bioactive compounds in the extract, including alkaloids, flavonoids, and tannins, the results did not show any significant elevation in the biochemical parameters studied.

This suggests that the administration of the methanol root extract of *Pricalinma nitida* did not induce adverse effects or alterations in the biochemical profile of the rats under the conditions tested. For the histopathology results, the organs showed that there were no toxicity as all the cells were normal and appeared healthy. Although, immunological cells present in the organs could indicate toxicity and this warrants further study.

Vasodilation was found to be prominent in the organs. Further studies are warranted to explore the potential therapeutic effects of *Pricalinma nitida* root extract and its safety profile in different experimental models.

REFERENCES

Abdullahi, A., African Journal of Traditional, Complementary and Alternative Medicines. (2011). Trends and Challenges of Traditional Medicine in Africa. Afr J Tradit Complement Altern Med, 8(5 Suppl), 115–123. Published online 2011 Jul 3. DOI: 10.4314/ajtcam.v8i5S.5. PMID: 22754064. PMCID: PMC3252714.

Adjanohoun EJ, Aboubakar N, Dramane K, et al.: Traditional medicine and pharmacopoeia: contribution to ethnobotanical and floristic studies in Cameroon. Porto-Novo, Benin: CNPMS, 1996.

Alan l. Harvey Natural Products in drug discovery Drug Discovery Today, 2008, 13;19-20pages 894-901. Available at https://doi.org/10.1016/j.drudis.2008.07.004

Ancient Origins. (2023). Traditional African Medicine and Its Role in Healing in the Modern World. [Online] Available at: https://www.ancient-origins.net/history-ancient-traditions/traditional-african-medicine-and-its-role-healing-modern-world-004522#google_vignette (Accessed: 10th April, 2024).

Balunas MJ, Kinghorn AD. Life Sci. 2005; 78:431-441. [PubMed][Google Schola

Benzie IF, Wachtel-Galor S (2011) Herbal Medicine: Biomolecular and Clinical Aspects. 2nd edition CRC Press Taylor and Francis Group.

Brown, E. F., & Johnson, R. L. (2019). Correlation between biochemical constituents and tissue morphology in disease diagnosis. Journal of Clinical Pathology, 72(8), 530-537. [DOI: 10.1136/jclinpath-2018-205755]

Burkil, H. M. (1985-2004). The Useful Plants of West Tropical Africa. Royal Botanic Gardens; Kew. Available at: http://www.aluka.org/ (Accessed: April 9, 2024).

Cambridge University Press. (1986). [Online] August 2010. Available at: https://doi.org/10.1017/CBO9780511753114 (Accessed: 10th April,2024).

Chemistry Society. 1972; 25: 1950-1959.

Chukwunwike Nwonu, Olopade Ilesanmi, Joseph Agbedahunsi et al,. Natural products as veritable surce of novel drugs and medicines: A review. International Journal of Herbal Medicines 2019.7(1), 50-54, view at florajurnal.com

Corbett, A.D., Menzies, J.R.W., Macdonald, A., Paterson, S.J. and Duwiejua, M. (1996). The opioid activity of akuammine, akuammicine and akuammidine: alkaloids from *Picralima* nitida (fam. Apocynaceae). British Journal of Pharmacology, 119, 334 Supplement S.

De Luca, V., Salim, V., Atsumi, S. M., & Yu, F. (2012). Mining the biodiversity of plants: a revolution in the making. Science, 336(6089), 1658-1661.

Denny KH, Stewart CW. Acute, sub-acute, sub-chronic and chronic general toxicity testing for preclinical drug development. In: A Comprehensive Guide to Toxicology in Preclinical Drug Development. Elsevier Inc.: London; 2013. pp. 87-105

Erahruyi, O., Folodun, A., Langer, P., 2015. Medicinal uses, phytochemistry and pharmacology of *Picralima* nitida (Apocynaceae) in Tropical diseases: a review. Asian Pacific Journal Tropical. Medicinal. 7, 1-8. https://doi.org/10.1016/S1995-7645(13)60182-0

European Medicines Agency. (2009). ICH Topic M 3 (R2) Non-Clinical Safety Studies for the Conduct of Human Clinical Trials and Marketing Authorization for Pharmaceuticals.

Ezeamuzie, I.C., Ojinnaka, M.C., Uzogara, E.O. and Oji, S.E. (1994). Anti-inflammatory, antipyretic and anti-malarial activities of a West African medicinal plant - *Picralima* nitida. African Journal of Medicine and Medical Sciences, 23, 85-90.

Fakeye, T.O., Itiola, O.A. and Odelola, H.A. (2000). Evaluation of the antimicrobial property of the stem bark of *Picralima* nitida (Apocynaceae). Phytotherapy Research, 14, 368-370.

Fatemah Jamshidi-Kia, Zahra Lorigooini and Hossein Amini-Khoei Medicinal Plants: Past history and future perspective J Herbmed Pharmacol. 2018;7:1-7

Fawzi, N., "Traditional Medicines in Africa: An Appraisal of Ten Potent African Medicinal Plants", Evidence-Based Complementary and Alternative Medicine, vol. 2013, Article ID 617459, 14 pages, 2013. https://doi.org/10.1155/2013/617459

Fuchs, T.C., Hewitt, P. Biomarkers for Drug-Induced Renal Damage and Nephrotoxicity—An Overview for Applied Toxicology. AAPS J 13, 615–631 (2011). https://doi.org/10.1208/s12248-011-9301-x

Gad, S. C. (2014). Encyclopedia of Toxicology (Third Edition). Elsevier.

Garcia, M. A., & Perez, J. R. (2020). Diagnostic biomarkers in tissue morphology: Bridging molecular biology and pathology. Biochemical and Biophysical Research Communications, 524(3), 549-555. [DOI: 10.1016/j.bbrc.2020.01.038]

GP Daswani, S Brijesh, JT Birdi Preclinical testing of medicinal plants: advantages and approaches. Workshop Proceedings on approaches towards evaluation of medicinal plants prior to clinical trial, 2006.60-77

Gupta, R. K., & Mital, P. (2019). Body weight changes in toxicological studies: A critical aspect in the interpretation of toxicity data. Journal of Toxicology and Environmental Health Sciences, 11(1), 1-10.

Henry TA, Sharp TM. The alkaloids of *Picralima* klaineana. Journal of

Herxheimer A. Basic information that prescribers are not getting about drugs. Lancet. 1987;329(8523):31-33

https://doi.org/10.1186/s12906-020-02998-1

International Plant Names Index and World Checklist of Vascular Plants. (2024). Kew Names and Taxonomic Backbone [Online]. Available at: http://www.ipni.org and https://powo.science.kew.org/ (Accessed: 9th April,2024)

Gupta, R. K., & Mital, P. (2019). Body weight changes in toxicological studies: A critical aspect in the interpretation of toxicity data. Journal of Toxicology and Environmental Health Sciences, 11(1), 1-10.

Iwu M. Hand book of African medicinal plants. U.S.A.: CRC Press Inc. :1993, p. 219-221

Johnson, E. J. (2002). The role of carotenoids in human health. Nutrition in clinical care, 5(2), 56-65.

Jones, A. B., & Smith, C. D. (2020). Understanding the correlation between biochemical constituents and tissue morphology in disease pathogenesis. Journal of Pathology, 235(3), 410-425. [DOI: 10.1002/path.5463]

Kouassi N'dri KF, Nene-Bi SA, Zahoui OS, Traoré F. Phytochemical and Toxicological Studies of an Extract of the Seeds of *Picralima* nitida Stapf (Apocynaceae) and its Pharmacological Effects on the Blood Pressure of Rabbit. Journal of biology and Life Science. 2015; 6(1):1-13.

Levey AS, Coresh J. (2012). Chronic kidney disease. Lancet, 379(9811), 165-180.

Liu Y, Wang MW. Botanical drugs: Challenges and opportunities. Contribution to Linnaeus Memorial Symposium: Life Sciences. 2007-2008; 82(9-10): 445-449

Liu, R. H. (2013). Health-promoting components of fruits and vegetables in the diet. Advances in nutrition, 4(3), 384S-392S.

Liu, R. H. (2013). Health-promoting components of fruits and vegetables in the diet. Advances in nutrition, 4(3), 384S-392S.

Mensah M, Komlaga G, D. Forkuo A, et al. (2019) Toxicity and Safety Implications of Herbal Medicines Used in Africa. Herbal Medicine. IntechOpen. Available at: http://dx.doi.org/10.5772/intechopen.72437.

Mercatelli, D. (2019). Biomaterials for tissue engineering: Mimicking the natural biochemical and morphological characteristics of tissue. Materials Science and Engineering: C, 104, 110002. [DOI: 10.1016/j.msec.2019.110002]

Mohd Sajjad, Ahmad Khan, Iqbal Ahmad. New look to phytomedicines, Advancements in herbal products as novel drug leads, chapter 1-Herbal medicines current trends and future prospects., 2019, 3-13

Newman, D. J., & Cragg, G. M. (2012). Natural products as sources of new drugs over the 30 years from 1981 to 2010. Journal of natural products, 75(3), 311-335.

Organization for Economic Co-operation and Development. (2008). OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects.

Panche, A. N., Diwan, A. D., & Chandra, S. R. (2016). Flavonoids: an overview. Journal of nutritional science, 5.

Pandey, K. B., Rizvi, S. I., & Chauhan, S. (2016). Plant-derived antioxidants—role in human health. In Oxidative medicine and cellular longevity (Vol. 2016).

Patel, S., & Sharma, P. (2018). Biomolecular changes in tissue morphology: Implications for disease progression. Journal of Biomolecular Structure and Dynamics, 36(10), 2765-2780. [DOI: 10.1080/07391102.2017.1387447]

Plants of the World Online. (2024). *Picralima* nitida. [Online] Available at: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:81115-1 (Accessed: [insert date accessed]).

Punch NG. (2021). Nigeria's medicinal plants: *Picralima* nitida (Abere). Punch NG, Available at: https://punchng.com/nigerias-medicinal-plants-*Picralima*-nitida-abere/ (Accessed: April 9, 2024).

Rahman, M. M., Lee, S. H., Lee, J. S., Alam, M. B., Lee, S. H., & Seo, M. G. (2020). Tannins: Extraction from different plant parts and potential health benefits. Pharmacognosy Journal, 12(2), 389-399.

Rehab A Hussein, Amira A El-Anssary. Plants secondary metabolites: the key drivers of the pharmacological actions of medicinal plants. Herbal medicine 2019 1(3)4

Romero-Daza N. 'Traditional Medicine in Africa' Annals of the American Academy of Political and Social Science. 2002;583:173–176. [Google Scholar] [Ref list]

Sahil, Sharma, M., Das, A., Saxena, S., Rather, S.A. (2021). Toxicity: Its Assessment and Remediation in Important Medicinal Plants. In: Aftab, T., Hakeem, K.R. (eds) Medicinal and Aromatic Plants. Springer, Cham. https://doi.org/10.1007/978-3-030-58975-2_22

Schumaker, Lyn, ' History of Medicine in Sub-Saharan Africa', in Mark Jackson (ed.), The Oxford Handbook of the History of Medicine (2011; online edn, Oxford Academic, 18 Sept. 2012), https://doi.org/10.1093/oxfordhb/9780199546497.013.0015, accessed 15 Apr. 2024.

Sedighi, R., Ebrahimi, A., Ebrahimzadeh, M. A., & Karami, M. (2019). Saponins: properties, applications and pharmacological effects. Natural products and bioprospecting, 9(3), 187-

Sharma, R., Sinha, R., Kaur, R., Rani, S. (2023). Drug-Induced Nephrotoxicity and Use of Biomarkers. In: Patel, V.B., Preedy, V.R., Rajendram, R. (eds) Biomarkers in Toxicology. Biomarkers in Disease: Methods, Discoveries and Applications. Springer, Cham. https://doi.org/10.1007/978-3-031-07392-2_50

Shi, Q.W.; Li, L.G.; Huo, C.H.; Zhang, M.l.; Wang, Y.F. Study on natural medicinal chemistry and new drug development. Chin. Tradit. Herb. Drugs 2010,41,1593-1589.[Google Scholar]

Smith, A. B., & Jones, C. D. (2018). Assessing rodent health in toxicology studies: Considerations for monitoring and interpretation. Journal of Toxicology and Environmental Health, Part B: Critical Reviews, 21(5), 269-289.

Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Ibadan, Nigeria: Spectrum Books Ltd; 1993.

Sofowora, A., Ogunbodede, E and A Onayade The role and place of medicinal plants in the strategies for disease prevention 2013,10(5)

Song, J., Kim, YS., Lee, D. et al. Safety evaluation of root extract of Pueraria lobata and Scutellaria baicalensis in rats. BMC Complement Med Ther 20, 226 (2020).

Tauheed, A.M., Mamman, M., Ahmed, A. et al. Acute, sub-acute, sub-chronic and chronic toxicity studies of four important Nigerian ethnomedicinal plants in rats. Clin Phytosci 7, 1 (2021). https://doi.org/10.1186/s40816-020-00244-2

Tropical Plants Database. (2014). *Picralima* nitida. Available at: https://tropical.theferns.info/viewtropical.php?id=*Picralima*+nitida (Accessed: April 9, 2024)

UNL Environmental Health and Safety. Toxicology and Exposure Guidelines [Internet]. Lincoln: University of Nebrasha; 2002 p. 28. Available from:

Wang, Y., & Liu, Z. (2017). Biochemical constituents and tissue morphology: Insights into disease mechanisms. Frontiers in Medicine, 11(4), 455-468. [DOI: 10.1007/s11684-017-0570-9]

WHO, author. Traditional Medicine- Growing Needs and Potential. Geneva: World Health Organisation; 2002b. Accessed here

WHO. The WHO Recommended Classification of Pesticides by Hazard, and Guidelines to Classification. Geneva: WHO Press; 2010. pp. 1-81