EVALUATION OF CREATININE AND UREA IN EXPERIMENTAL RATS FED THE AQUEOUS FRUIT PULP EXTRACT OF OSU (PICRALIMA NITIDA)

BY

Osagioduwa Joseph AJAYI

BMS1802338

A PROJECT WORK SUBMITTED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, SCHOOL OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.SC) IN MEDICAL BIOCHEMISTRY.

OCTOBER, 2023

CERTIFICATION

We the undersigned certify that Ajayi Osagioduwa Joseph presented this seminar paper to the Department of Medical Biochemistry, School of Basics Medical Sciences, University of Benin.

Signed : …… ……

Professor (Mrs) H.A. OBOH Date

(Project Supervisor)

…… …….

Dr. F. Olumese Date

(Head of Department)

…… **…….**

External Examiner **Date**

DEDICATION

This project is dedicated to God Almighty and to my late mother of blessed memory, Mrs Caroline Ajayi, My father Mr. S.J Ajayi and to my siblings for their immense support throughout my academic journey.

ACKNOWLEDGEMENTS

I am grateful to God for the successful completion of undergraduate's journey. My gratitude goes to my biological father Mr. S.J Ajayi and to my siblings for their immense support throughout my academic journey. Special appreciation goes to elder brother, Mr Felix Igbinijesu for your tireless sacrifices toward this my academic programme, I will not fail to mention Mr Frederick Igbinijesu, who was also very instrumental during my program

Special thanks to my Supervisor Prof. Henrietta A. Oboh for her unfailing and untiring guidance, support, useful suggestions and constructive criticism with worthwhile comments that brought about the completion of this project work. Certainly I'm not forgetting my project colleagues and friends Julius, Mackay, Faith, Paul, Christain, Flourish, Enoch, Uhunoma, Rosella. Thank you all for coming through for me. I'm indeed grateful to my very good friends Mr. Martins Olu Ayode, Dr Victory Adegboye, Marvellous my course rep, My course adviser Dr Aguebor –Ogie Ngiomwan. The Head of department, Dr F. Olumese, and other academic staff for their contributions; Dr Anionye, Mr Aisosa, Mr Igiebor, Mr Ododo, only to mention a few.

TABLE OF CONTENT

ABSTRACT

This study was designed to evaluate the creatinine and urea values in experimental rats fed with aqueous fruit pulp extract of *Piralima nitida (OSU)*. The expense of the orthodox drugs has led to the increase demand for medicinal plants that are effective to treat diverse ailment. Serum urea and creatinine are widely accepted parameters to assess chronic kidney disease status as well as to assess renal status in susceptible diabetic and hypertensive subjects. Urea and creatinine are nitrogenous end products of metabolism. The effect of daily intake of the aqueous unripe fruit pulp of P. nitida on renal function was studied. Six groups of five (5) rats each were distributed according to weight (average body weight 135.0-185.0g). The test groups received aqueous fruit pulp extract of *Picralima nitida* dissolved in distilled water at 200, 500, 1000, 2000 and 3000 mg/kg body weight/day/rat orally using gastric gavage. The normal control animals had distilled water *ad libitum*. The animals were observed for signs of toxicity and mortality throughout the experimental period. The weight and the feed consumed by the rats were measured weekly during the feeding trial with a weighing balance. At the 35th day, the animals were fasted for 12 hrs and euthanized by decapitation. Blood was collected in appropriate containers for biochemical evaluation. The serum from each group was used to determine the levels of alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], and total protein [Randox] using commercial kits according to the manufacturer's direction. Creatinine and urea levels in the treated groups were not significantly ($p > 0,05$) altered in the treated groups when compared to control. This means the Kidney is performing optimally well, these no disease condition attached to it.

CHAPTER ONE

INTRODUCTION

1.0 Background of the study.

Medicinal plants, known as Akuamma seeds, are found in Côte d'Ivoire, Uganda, DR Congo, Cabinda, and West Africa, and have been proven to treat various ailments and alleviate disease-related discomfort.

It is estimated that this plant grows between 12 and 30 meters tall, with a girth of between 0.6 to 1 meters. Its bark is grayish; the slash is fibrous, yellowish with dark flecks, and produces a sparse amount of latex along with other blackish branchlets. The blades of the leaves are rounded to slightly cunneate, and they are broadly oblong-elliptic to oblong-lanceolate. They are also shortly acuminate. They have a glossy, glabrous, leathery, dark green color. There are several thin, parallel nerves with narrower veins in between. It bears terminal inflorescences of up to 5 cm broad white blooms. The calyx, which has five overlapped triangular-ovate lobes, is roughly 2.5 cm long and has a large cup-shaped base. The entire inflorescence is glabrous. Five elliptic flower lobes are seen in the thin, 2.5 cm long corolla tube. (*Ilobi, et al., 1990*). Usually seen in pairs, the fruits dangle at the end of a lengthy stem. They have a large oval shape, are smooth, glabrous, and are green in color when mature, becoming yellow or orange. They have many seeds (5–10 cm broad) buried in a pulp and range in size greatly, up to 15 cm long and 10 cm wide. The plant is quite bitter throughout.
Ethnomedicinal Uses: Various fever and malaria therapies are prepared using preparations of the seeds, leaves, or stem bark. It is used to treat jaundice, malaria, and primary high blood pressure in Central Africa. Pneumonia and stomachaches are treated with the pulverized seeds. Seed and stem bark extracts are used as a vermifuge and to treat genital disorders. Akuamma seeds, stem bark, and dried fruit rind are highly valued in medicinal markets in

Ghana, Nigeria, and Ivory Coast for their antimalarial and sleeping sickness-relieving properties. The seeds are utilized to make a remedy for mental health conditions and associated CNS disorders.. The root decoction is dispensed as a cure for yellow fever. (Iwu *et al*., 1981)

Constituents — The plant elaborates a complex mixture of indole and dihyroindole alkaloids, including alstonine, akuammiline, akuammidine, akuammine, akuammigine, akuammicine, echitamine, picraline, picratidine, and picraphylline. (Sokoloski *et al*.,1990)

Pharmacological Studies: It has been established that plant extracts have sympatholytic, .opiate receptor, local anesthetic, and antihypertensive effects. AKAammidine, AKAammine, and AKAammigine are three of the main indole alkaloids found in the plant. The leaves elaborate different indole bases, notably picraphylline and picraline. Akuammidine has a sympatholytic action and a hypotensive effect, which was reported to be weaker than that of yohimbine.(Raymond *et al*.,1951) The compound also showed strong local anesthetic action, which was found to be three times that of cocaine hydrochloride. (Sokoloski *et al*., 1990) The aqueous decoction of the bark has been shown to be active against *Trypanosoma brucei*. (Wosu *et al* 1989). The in vitro efficaciousness of P. nitida fruits' primary alkaloids against drug-resistant and -sensitive strains of Plasmodium falciparum has been investigated. With IC50 values ranging from 0.08 to 0.9 μg/ml, the alkaloids exhibited impressive inhibitory activity against both P. falciparum clones. The compounds with the highest activity were those of the akuammicine type, and those in the picraline-akuammine subgroup. The alkaloid echitamine was inactive. (Iwu *et al*., 1991). Powerful inhibitory activity of the alkaloids was also shown against clinical isolates of trypanosomes and Leishmania.

Picralima is currently used as a crude extract in traditional African medicine as an antimalarial and for blood pressure control, even though its exact mode of action is still unknown.

. Alstonine, found in the seeds and fruit rind, has shown antipsychotic-like effects, a putative antipsychotic, which consistently differed from the effects of known drugs in various mouse models (Elizabetsky *et al*., 2006; Herrman *et al*., 2008) Treatment with alstonine was able to prevent MK801-induced working memory deficit, indicating its potential benefit for cognitive deficits now seen as a core symptom in the disease. As previously noted, alstonine was also successful in reversing the hyperlocomotion and social interaction deficit caused by MK801. Ritanserin, a 5-$HT_{2A/C}$ receptor antagonist, prevented alstonine's effects on these three behavioral parameters. (Herrman *et al*., 2008).

Additionally, Picralima nitida has been employed as a natural sedative to promote relaxation and alleviate anxiety. It has been integrated into cultural practices to enhance mood, reduce stress, and support restful sleep; it also helps in finding out whether P. nitida possesses any hypoglycemic effect as well as it's effect on other body organs (Elizabetsky *et al*., 2006).

1.1 JUSTIFICATION OF THE STUDY

The herbal remedy Picralima nitida possesses a great deal of promise, worth, and untapped sources of powerful drugs for disease management. Strong reports on the effectiveness of P.nitida extracts from the fruit pulp, bark, seeds, and roots can be found in the literature.. However, there is paucity of information about the effect of P.nitida fruit pulp on the kidney function in experimental rats.

Picralima nitida, also referred to as "Osu" or "Akuamma," is indigenous to West Africa. The fruit of the Osu plant, namely its seeds, are prized for their therapeutic qualities and have historically been utilized in African folk medicine. Alkaloids found in the seeds have been

linked to pharmacological activities such analgesic, anti-inflammatory, and antimalarial characteristics. The 'Osu' fruit's aqueous fruit pulp extract is of interest because of its reputed health advantages and historical application. The safety of herbal medicine use has recently been questioned due to reports of illness and fatalities particularly nephrotoxicity (Park *et al*., 2010). Consumers continue to have doubts about the efficacy and safety of many traditional herbal medicines, even though only a small number have undergone clinical trials to confirm their effectiveness.

1.2. Objective of the Study:

The study aims to assess the impact of creatinine and urea on experimental rats that are given an aqueous extract of the unripe fruit pulp of *Picralima nitida*, also known as "Osu."

CHAPTER TWO

LITERATURE REVIEW

2.0 Picralima nitida

Tropical Picralima nitida, sometimes called "Osu" or "Akuamma," is a plant that is indigenous to West Africa. It is a member of the Apocynaceae family, which contains numerous plants having therapeutic effects.The tropical rainforests of West Africa are the natural habitat of Picralima nitida. Nigeria, Ghana, Sierra Leone, Liberia, the Ivory Coast, and Cameroon are among the nations where it is frequently seen. Osu, for instance, is prevalent in southern Nigeria, particularly in the Niger Delta and the rainforests of the southeast states.

The Osu tree is an evergreen tree of medium size that can grow up to 25 meters in height. Large, glossy leaves with a leathery texture grow from a straight trunk with grayish bark. Round, green fruits that eventually turn yellow or orange are produced by the tree. Five to six seeds, the major components used for therapeutic purposes, are usually found inside the fruit.The Osu tree's seeds, bark, leaves, and roots, as well as other parts, have all been used in traditional African medicine, to treat a range of illnesses. For their therapeutic qualities, the seeds in particular are highly prized. They are frequently used as a natural medicine for treating problems like pain relief, malaria, digestive issues, and other conditions.

Figure 1. Picralima nitida fruit

Figure 2: Picralima nitida leaf

2.1PHYTOCHEMISTRY OF PICRALIMA NITIDA

Picralima nitida, also referred to as "Osu" or "Akuamma," is a tropical plant that has undergone extensive pharmacological research. The chemical components and reactions that take place in Picralima nitida when it is exposed to light are referred to as its photochemistry. Research on Picralima nitida's chemical makeup sheds light on potential photochemical transformations that might take place, despite the paucity of studies specifically on the photochemistry of this plant.(Fakeye *et al*., 2000).

Picralima nitida is known to contain various classes of compounds, including alkaloids, flavonoids, terpenoids, and phenolics. The alkaloids, in particular, have received significant attention due to their diverse pharmacological activities.

The indole alkaloids akuammine, akuammicine (strychnan class), akuammidine and akuammiline (both corynanthean class), akuammigine, and the very similar alstonine, pseudo-akuammigine, and picraline are the main compounds found in the stem bark, fruit, and seeds of Picralima nitida. The mature seeds contain akuammine as the primary alkaloid, along with pseudo-akuammicine, picranitine, picratidine (N-methylpicraline), eburnamine (desacetylpicraline), and desacetylakuammiline (rhazimol). The seeds are especially rich in alkaloids (3.5–4.8%). Acupammigine, Acupammicine, Picracine, and Desacetylpicraline are found in the root bark, while Acupammine, Acupammigine, Picraphylline, and Melinonine A are found in the leaves. There is also picracine in the stem bark.

Strong sympathomimetic and local analgesic properties of akuammine are similar to those of cocaine. It doesn't affect breathing, but it causes dogs to have noticeable, long-lasting hypotension. It has a strong inhibitory effect on intestinal peristaltic movements at higher doses. At such doses it also has hypertensive activity with a weaker, but longer lasting effect than yohimbine (Corbett *et al*., 1996).

Acauammigine demonstrates pronounced sympatholytic action and counteracts the effects of adrenaline on the heart, blood vessels, and circulatory system's regulatory center. Akuammidine exhibits skeletal muscle relaxant, hypotensive, and local anesthetic properties. It has roughly three times the potency of cocaine as an anesthetic for local use. It has a selective sympatholytic action without concurrent parasympatholytic effects. It opposes akuammine and decreases the sympathetic nervous system's irritability. As an inverse, competitive, reversible parasympathomimetic, pseudo-akuammigine functions. It stimulates the central nervous system, respiration, skeletal muscle contraction, and smooth contractions of muscles at low and high doses, respectively. It has local analgesic, anti-inflammatory, cholinesterase-inhibiting, and hypotensive properties. It also lengthens the time that hexobarbital induces sleep. Only found in Picralima nitida cell suspension cultures, pericine and pericalline demonstrated in vitro opium-antagonist activity. (Menzies *et al*., 1998).

Alstonine has demonstrated antipsychotic medication effects in mouse experiments for the treatment of schizophrenia, without some of the negative effects associated with the widely used medication clozapine. Alstonine doesn't seem to have clozapine's proconvulsant qualities.

Rats administered extracts from the seeds exhibit analgesic effects similar to those of morphine. The alkaloids akuammidine, akuammine, pseudo-akuammigine, and akuammicine have been shown to exhibit agonist and antagonist activities at opioid receptors to differing degrees in vitro. The opioid bioassays demonstrated minimal or no efficacy for akuammigine.. Since the opioid receptors interact to mediate the analgesic effects, it is important to look into the possibility of addiction and dependence.. The seed extracts showed also significant anti-inflammatory activities in several rat models (Ramirez *et al*., 2003).

Even at low concentrations, the extracts of fruit rind, stem bark, and roots demonstrated significant inhibitory effects against strains of Plasmodium falciparum which were resistant

to chloroquine in vitro. The fruit rind's dichloromethane extract was the most powerful. The antimalarial activity is also present in the seeds and leaves, but at a lower level (Neuwinger *et al*., 1996). When applied to Plasmodium strains resistant to chloroquine, akuammine exhibited only modest antimalarial activity. There was limited activity against gram-negative bacteria, but significant antimicrobial activity against a wide range of gram-positive bacteria and fungi was shown by the basic fraction of the stem bark methanol extract. The basic fraction exhibited lower minimum inhibitory concentrations (MIC) against Aspergillus flavus and Aspergillus niger than tiaconazole, and a similar MIC for Staphylococcus aureus as the control medication ampicillin. A methanol extract of Picralima nitida stem bark was used in a cream formulation that demonstrated remarkable efficacy in treating skin conditions such as athlete's foot, tinea pedis interdigitalis, tinea corporis, and pityriasis versicolor in clinical trials. At concentrations of 50 μg/ml or less, a stem bark methanol extract was also found to be effective against a visceral Leishmania isolate. Trypanosoma brucei was significantly inhibited by a hot water extract of the stem bark, which was statistically comparable to the effects of diminazene aceturate (Berenil), a drug that is frequently used to treat sleeping sickness. By a mechanism unrelated to the availability of insulin from pancreatic β-cells, bark and seed extracts produced hypoglycemia in both normal and alloxan-induced diabetic rabbits. The standard medication tolbutamide was slower to exhibit hypoglycemic action than the seed extract. Acute toxicity tests in rats showed a dose-dependent acute intraperitoneal toxicity (Francois *et al*., 1996)

The wood is pale yellow, hard and elastic, and polishes and finishes well.

2.2 Description of PICRALIMA NITIDA

P. nitida is a 35 m high shrub with glabrous, white latex covering all surfaces; it has a 60 cm length bole; the bark is hard and brittle, ranging from dull to dark greyish black or brown, seamless to slightly rough, or finely striped. The leaves are opposite, simple, and entire. There are no stipules, and the petiole is 1-2 cm long. The blade is elliptical to oblong, measuring (5–)10–26 cm × 2–13 cm, with a cuneiform base and an abruptly acuminate apex. It is pinnately veined, in 14–23 connects of lateral veins. The inflorescence is a compound, umbel-like cyme that is 6–10 cm long and has 10–35 flowers. The peduncle is 2–35 mm long and has three main branches. The bracts are very small. Flowers: pedicel 2–20 mm long; sepals almost free, imbricate, broadly ovate to almost orbicular, 5–7 mm long; corolla with soft elongated tube; bisexual, regular, 5-merous, fragrant or not, open during the day. 25–45 mm long, hairy inside, and enlarging below the stamen insertion; lobes ovate, 14–30 mm × 6–10 mm, apex obtuse, spreading or erect, white to yellow; stamens inserted above the corolla tube, included; anthers ovate, 3–4 mm long; ovary superior, consisting of two separate carpels, united at the extreme base by a disk-like thickening; pistil head with an oblong basal part and a filiform stigmoid apex up to 1.5 mm long. Fruits are composed of two free, smooth, rounded, oblong to ellipsoid, 11–20 cm long, two-valved, several–to–many seeded follicles that are yellow–orange in color. The seeds are flattened, oblong to obliquely ovate, 2.5–4.5 cm long, smooth, brown to orange, and embedded in a soft pulp that ranges in color from white to orange. Epigeal germination seedling with oblong to oblong or ovate cotyledons, 10–13 mm long, slightly cordate to rounded base, and obtuse to rounded apex (Bulkill *et al*., 1985)

2.3 USES OF PICRALIMA NITIDA

Picralima nitida, commonly known as 'Osu' or 'Akuamma,' has a long history of traditional use in African folk medicine for various purposes. The plant's various parts, such as the seeds,

bark, and leaves, are used for their therapeutic qualities.. Here are some of the traditional and potential uses of Picralima nitida;

1. STEM BARK

Analgesic Properties: The stem bark of Picralima nitida is known for its analgesic or pain-relieving properties. It has been traditionally used to alleviate different types of pain, such as headaches, toothaches, and muscular pain.

Antimicrobial Activity: Studies have indicated that the stem bark of Osu possesses antimicrobial properties. It may be used to combat certain bacterial and fungal infections.The basic fraction (BF) of the methanol extract of the stem bark of Picralima nitida (family Apocynaceae) exhibited significant antimicrobial activity against a wide range of Gram-positive bacteria and fungi, but limited activity against Gram-negative bacteria (Fakeye *et.al*; 2000)

2. LEAVES

The aqueous leaf extract of Picralima nitida has been shown to produce antipyretic and anti-inflammatory effects in the management of several disease conditions, including diabetes (Folorunso *et.al;* 2022)

3. SEEDS

For decades, individuals made use of the seeds of the Picralima nitida akuamma tree to alleviate fever and pain.. Previous studies have attributed these effects to a series of indole alkaloids found within the seed extracts (Creed *et.al* 2020). Picralima nitida seeds are valued in traditional medicine as good substitute for quinine in treatment of malaria,cough

depressant,chest complaints, including pneumonia, anodyne for tropical injuries and also as enema (Burkhil,1985).

4. FRUITS

Traditional Medicine: The fruit of Picralima nitida is employed for a number of reasons in conventional medicine;

Analgesic and Antipyretic Properties: The fruit is believed to have analgesic properties, offering pain relief, and it may also help reduce fever.

Aphrodisiac Effects: In traditional practices, Osu fruit is considered to have aphrodisiac properties and is used to enhance sexual vigor.

Ritual and Cultural Significance: The fruit holds cultural and spiritual significance in certain regions and is used in religious ceremonies and rituals.

A preliminary pharmacological screening of the methanolic extract of Picralima nitida fruit showed potent and dose-dependent anti-inflammatory, antipyretic and anti-malarial activities (Ezeamuzie et.*al 1994)*

2.4 BIOLOGICAL ACTIVITY OF PICRALIMA NITIDA

Picralima nitida, commonly known as 'Osu' or 'Akuamma,' exhibits a range of biological activities that have been the focus of scientific research. The plant contains several bioactive compounds, including alkaloids, flavonoids, terpenoids, and phenolics, which contribute to its

pharmacological properties. Here are some of the notable biological activities associated with Picralima nitida:

1. Analgesic activity: Picralima nitida has long been utilized as a herbal analgesic. In numerous investigations, the plant's alkaloids, including akuammidine, akuammine, and akuammicine, have demonstrated analgesic properties. These substances relieve pain by interacting with opioid receptors in the central nervous system.

2. Anti-inflammatory activity: Research has shown that Picralima nitida extracts and compounds have anti-inflammatory activities. Inflammation is decreased by the plant's alkaloids, especially akuammidine and akuammicine, which have inhibitory effects on inflammatory mediators and cytokines.

3. Antibacterial activity: Extracts from Picralima nitida have demonstrated antibacterial action against a number of microorganisms, like fungi and bacteria. The plant's constituents may prevent pathogenic germs from multiplying and growing, demonstrating its potential as natural antimicrobial agents.

4. Antioxidant activity: Picralima nitida is rich in phenolic compounds and flavonoids, which possess potent antioxidant properties. These compounds scavenge oxidative stress, lowering free radicals, and shielding cells from harm. The activity of antioxidants of Picralima nitida contributes to its potential in combating oxidative stress-related diseases.

5. Antimalarial activity: Some studies have reported the antimalarial activity of Picralima nitida extracts and alkaloids. The plant's compounds, such as akuammidine and akuammigine, exhibit inhibitory effects against Plasmodium falciparum, the parasite responsible for malaria. This suggests a potential role for Picralima nitida in the development of antimalarial treatments.

6.Anti-diabetic activity: Studies have suggested that Picralima nitida possesses anti-diabetic properties. Compounds from the plant have been shown to boost glucose absorption, increase

insulin production, and regulate blood sugar levels, all of which may help with the management of diabetes.

2.5 Evaluation of Creatinine and Urea

2.5.1 Creatinine

The quantity of creatinine that is in the blood can be measured by the creatinine blood test. The objective of this test is to gauge kidney function.

Urine testing is another method for measuring creatinine

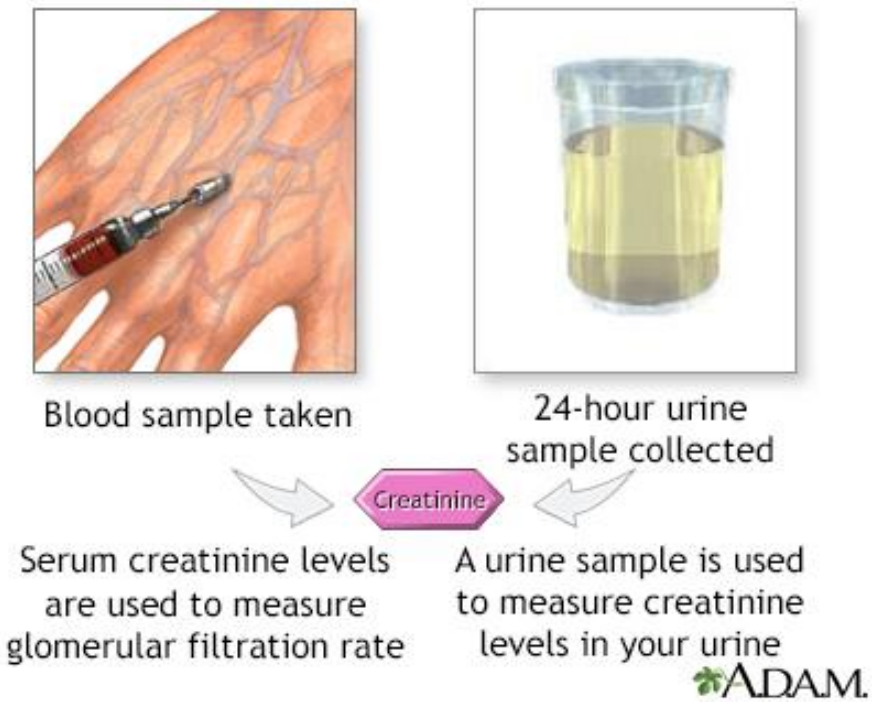


Figure 3. Creatinine.

One of creatine's chemical byproducts is creatinine. The body produces the chemical creatine, which is primarily used to fuel muscles. The purpose of this test is to evaluate kidney function. The kidneys eliminate creatinine from the body completely. Your blood's level of

creatinine will rise if your kidney function is abnormal. This is because less creatinine is excreted through your urine. (Landry *et al*., 2020)

Normal Results

For men, the normal result ranges from 0.7 and 1.3 mg/dL (61.9 to 114.9 µmol/L) and for women, among 0.6 and 1.1 mg/dL (53 to 97.2 µmol/L).

Compared to men, women typically have lower creatinine levels. This is a result of women typically having smaller muscle mass than men. The size and mass of a person's muscles affect their level of creatinine.

A measurement when assessing kidney function, the serum creatinine level is frequently utilized. Urine creatinine levels can be part of the creatinine clearance test or used as a screening test to assess kidney function.

These medicines include:

Cimetidine, famotidine, and ranitidine

Certain antibiotics, such as cefoxitin and trimethoprim (Landry *et al.,* 2020).

Common measurements for test results are shown in the above examples. The normal value ranges in various laboratories may differ slightly. Certain labs test different samples or employ different measurements.

What Abnormal Results Mean

The following conditions could cause a higher-than-normal level:

- Blockage of the urinary tract;
- Kidney issues, such as kidney damage or failure, infection, or decreased blood flow

- Fluid loss from the body (dehydration)
- Issues with muscles, such as rhabdomyolysis, or the disintegration of muscle fibers.

  Problems during pregnancy, such as seizures caused by eclampsia or high blood pressure caused by preeclampsia

A lower than normal level may be due to:

- Nerve and muscle disorders that result in a loss of muscle mass
- Food Insecurity.

  There are many other conditions for which the test may be ordered, such as high blood pressure, diabetes, or medicine overdose.(McPherson *et al*., 2022)

2.5.1.1 Risk factor for Serum Creatinine

There is little risk involved with having your blood taken. The size of arteries and veins varies from person to person and from side to side of the body. It might be harder to draw blood from some people than from others.

Although they are minimal, additional risks connected to getting blood drawn include:

- Long-term bleeding
- Loss of consciousness or dizziness
- Several punctures to find veins
- Hematoma, or collection of blood beneath the skin
- Infection (a small risk whenever skin breaks)

2.5.2. UREA

Urine is mostly made up of urea, an organic substance that is created during the breakdown of nitrogen and protein. It is used in a variety of products, such as creams for the treatment of skin conditions like psoriasis, eczema, atopic dermatitis, and ingrown nails, as well as fertilizer and other industrial processes.

Some people have problems with the urea cycle, which prevents them from getting rid of waste products properly and can cause neurological symptoms.

Find out more about the urea production process in the body, its significance, its applications, and the conditions that affect it.

Figure 5. Urea Production.

The urea cycle, a biochemical mechanism by which the body gets rid of waste, produces urea. Proteins from diets high in excess or from aging cells that need to be replaced must be broken down on a regular basis. Ammonia is a poison that is produced when proteins break down and is unsafe for humans to safely expel.

Rather, we employ a series of steps involving multiple proteins to transform ammonia into less toxic urea. This process occurs in the liver; then, urea is released into the bloodstream, where it travels to the kidneys and is finally excreted as urine. (Rebecca *et al*., 2011)

Blood Urea Nitrogen (BUN)

Renal function can be assessed with a routine blood test called blood urea nitrogen (BUN). This test gauges blood levels of waste from cell metabolism by measuring the amount of nitrogen from urea. Increased or decreased BUN can indicate a number of illnesses, such as liver or kidney diseases.

BUN levels can also be impacted by other elements like food, hydration, and medication.. Therefore, it is important to look at BUN levels in conjunction with other tests, such as creatinine (waste level tests), to get an accurate picture of kidney function. (Rebecca *et al*., 2011)

Urea Cycle Disorders

A urea cycle disorder can occasionally be brought on by one or more flaws in the proteins involved in the urea cycle. When this occurs, the toxic ammonia waste that is produced by the breakdown of proteins is not adequately processed by the body.

Causes

Urea cycle disorders are caused by an abnormal gene inherited from one or both parents and typically receive diagnosis in infancy or childhood. One of the two protein transporters or any one of the six enzymes that regulate the urea cycle can be defective in a person with a urea cycle disarray

Symptoms

The body can experience mild to severe symptoms based on which enzymes are affected by the accumulation of ammonia. Depending on the severity of the defects, the symptoms of

urea cycle disorders can manifest in infancy or early childhood. These disorders primarily affect the brain and nervous system. Early infancy symptoms that could manifest include

- Lethargy or sleepiness
- Refusal to eat or vomiting
- Low body temperature (hypothermia)
- Convulsions
- Posturing (rigidity) of muscles
- Coma
- A respiratory arrest

In less severe cases, symptoms might not appear for months or years after birth, and ammonia levels do not rise as quickly. Symptoms in these situations could include:

- Problems with behavior or mental health;
- Convulsions
- Avoidance of foods with high protein content
- Vertigo and queasines. (Rebecca *et al*., 2011)

Urea Uses

Urea is used in many ways outside of the human body. Because of its high nitrogen content, it is utilized as a fertilizer in agriculture. It is used in cold packs, animal feed, barbituric acid, and other products. It is also used in other industries.1.

Dermatologists also frequently use urea as a topical cream to treat a variety of skin conditions, including seborrheic dermatitis, ichthyosis, eczema, psoriasis, and xerosis. This is partially

due to the fact that urea is a molecule that can absorb water, a property known as hygroscopicity.

Additionally, urea strengthens the skin barrier and possesses antimicrobial qualities.[5]

Urea is an organic compound that is the main component of urine.

It is a safe option for treating many skin conditions that cause scaly, dry skin because it is one of the most popular moisturizing agents and has few mild side effects.

Urine mostly consists of an organic compound called urea. It is created when ammonia, a less toxic substance that the body can get rid of, is produced when proteins break down. Urea cycle disorders, which result in an accumulation of ammonia and severe symptoms like neurological damage, can be caused by specific genetic defects. Urea has other uses outside the body, such as in nitrogen-rich fertilizer in agriculture and moisturizing topical cream in dermatology. (Rebecca *et al*., 2011)

CHAPTER THREE

MATERIALS AND METHOD

3.1 Plant material

The unripe fruits of *Picralima nitida* was obtained from a local vendor in New Benin market, New Benin, Benin City, Nigeria. The fruit was recognized and confirmed by Dr. Henry Akinnibosun, a Taxonomist at the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria. After obtaining it, a voucher with the number UBHP 424 was placed inside the Herbarium. Mud and contaminants were removed from the P. nitida fruits by giving them a thorough wash in clean water from the surfaces and left to dry overnight in a sieve, after which the green rind was peeled and separated from the pulp. The pulp were also seperated from the seeds used a kitchen knife to finely chop the mixture into small, two-centimeter pieces. The weight of the pulp was 20Kg.

3.2 Preparation of the crude extract

The chopped *P. nitida* fruit pulp was placed on a flat surface and air-dried at room temperature (25^0C) over the course of 56 days, to a steady weight. The dehydrated fruit pulp was pulverized to powder using a mechanical grinder [model: SY-18B Industrial Dry Herbs Grinder, China]. The powdered fruit pulp (5.01kg) was soaked in distilled water for 72 hours at a mass to volume ratio of 1: 10 (kg/L) with continuous stirring. The solution was filtered through muslin cloth, cotton wool and filter paper. The filtrate obtained was freeze dried [Biobase BK-FD10 Freeze Dryer, China] producing a concentrated jelly-like extract. A total yield of 637.6g (12.7%) was obtained. The clean, airtight plastic containers containing the freeze-dried extract were kept at 4°C until they were needed.

3.3 Apparatus.

Weighing balance (Adventurer: OHAUS CORP, China), Measuring cylinder, Beakers, Test tubes, Stirrer, Micropipette, Shaking water bath (Jinotech, SHZ-82, China), Heating drying oven (Model DHG, Mermert Industries, Germany), Spectrophotometer (721S Visible Spectrophotometer, China), mpact drill (13mm, JEMENS, Germany), Bucket Centrifuge (90-2 electric low-speed centrifuge, China), Hot water bath, Freeze Dryer (Search-tech, British), Incubator (Seradon), Knives, Plastic Bowls, Muslin cloth, Cotton wool, Filter paper, Sterilized or oven dried containers, Filter paper, Foil paper, Gloves, Handkerchief, macro or Micro pippete, Racks, Refrigerator, Spatula, Test tube racks, Funnel, Conical flasks, Cuvette, Washing bottles, Washing brush, 100ml volumetary flask, Bin bags, Tissue paper, Gallon (10litre), Rubber tray, Ice packs.

3.4 EXPERIMENTAL ANIMALS

Average-weight adult male Wistar rats in good health (180-220g) were obtained from the animal house situated at the Department of Anatomy, University of Benin, Benin City, Nigeria. Approval for the study was obtained after a review of the protocol by the Ethical Committee, College of Medical Sciences, University of Benin, Benin City, Nigeria, with number CMS/REC/2022/281. They were maintained according to the guidelines of Committee for the Purpose of Control and Supervision of experiments on animals (National Research Council, 1997). The animals were kept in hygienic plastic cages with appropriate bedding made of wood shavings and a 12-hour light/dark cycle. The bedding was changed every day, and the animals were sorted by weight. They were fed with pelletized poultry finisher's mash feed (Top Feeds, Ibadan, Nigeria), identified using permanent markers on their tails and allowed to acclimatize for 14-days.

3.5 Animal grouping

For phases I, II, and III of the experiment, the rats were split into distinct groups**.**

Acute toxicity

Phase I

The rats were divided into 4 groups, three rats per group. Group 1 was the control group, group 2, 3 and 4 received 10mg, 100mg and 1000mg/kg body weight respectively for 1 day (24hours).

Phase II (LD_{50})

The rats were divided into 4 groups, one rats per group. Group 1 was the control group, group 2, 3 and 4 received 1600mg, 2900mg and 5000mg/kg body weight respectively for 14 days.

Subchronic toxicity

Phase III

The rats were divided into 6 groups, five rats per group. Group 1 was the control group, group 2, 3, 4, 5 and 6 received 200mg, 500mg, 1000mg, 2000mg and 3000mg/kg body weight respectively for 35 days.

3.6 Apparatus

- Centrifuge (model: D-7200), Test tubes racks, Electronic balance, Spatula, gastric gavage, test tubes, Dissecting board, Micropipettes, Plain sample containers, Refrigerator, Dissecting kit, Syringes and needles, Visible Spectrophotometer (model: 721), Conical flask, Beaker, Glass funnel, Measuring cylinder, Surgical blade, Masking tape, Permanent markers, Freeze dryer (Biobase BK-FD10 Freeze Dryer, China), Shaking water bath (Jinotech, SHZ-82, China), Face masks, Measuring cylinder, Weighing balance (Adventurer: OHAUS CORP, China), Latex hand gloves, Filter paper, mechanical grinder, air-tight clean glass container, Heating drying oven (DHG model), Muslin cloth, stop watch, pipette, freeze dryer, desiccators and its guaze, plastic containers.

3.7 Experimental Procedures and Design

3.7.1 Acute oral toxicity

The evaluation of acute oral toxicity of *Picralima nitida* aqueous fruit pulp extract was carried out using Lorke's method (Lorke, 1983). Sixteen (16) rats were used for this study and involved two phases; in phase I, there were three treatment groups with three rats per group as against control group that had distilled water. The extract was administered as follows: group I (normal rats that received distilled water), groups II, III and IV were orally administered 10 mg, 100 mg and 1000 mg/kg body weight of aqueous fruit pulp extract of *Picralima nitida* respectively in a single dose using gastric gavage. In phase II (LD_{50}), there were three treatment groups of one animal each (group V, VI and VII), and were orally administered 1600 mg, 2900 mg and 5000 mg/kg body weight of extract respectively in a single dose using gastric gavage. For fourteen days, at least once a day, the animals were observed for behavioral changes, signs of gross toxicity, and mortality one hour after dosing. Before and after the observation period, body weights and feed intake were noted.

3.7.2 Subchronic toxicity testing

Based on average body weight (135.0–185.0g), six groups of five (5) animals each were assigned. The aqueous fruit pulp extract of Picralima nitida was given daily for 35 days to determine the subchronic toxicity study. Using gastric gavage, the test groups were given an aqueous fruit pulp extract of Picralima nitida dissolved in distilled water at 200, 500, 1000, 2000, and 3000 mg/kg body weight/day/rat. Distilled water was available to the standard control animals at all times. Throughout the course of the experiment, the animals were watched for indications of toxicity and mortality. Using a weighing balance, the weight and amount of feed that the rats consumed were recorded every week during the feeding trial. The animals were decapitated and fasted for 12 hours on the 35th day before being put to death. For biochemical analysis, blood was drawn into the proper containers. The pancreas, kidney,

heart, lungs, spleen, and liver were removed, their adherent tissues removed, and they were weighed, and kept in plain bottles containing 10% buffered formalin for histopathological assessment.

3.8. **Blood Glucose Analysis**

Fasting blood glucose was determined in all the groups using a glucometer [ACCU-Check, Roche, Germany] on day 0, 3, 6, 9, 12, 15, 18, 21, 24, 27, and 35. Blood glucose levels were measured after blood samples were taken from the rats' tips of tails. Using the formula, the percentage glycemic change at any given time was determined:

$$\% \text{ G lycemic change} = \frac{GC - FBG}{FBG} \times 10\,0$$

Where: GC is the glucose concentration at different time points and FBG is the fasting blood glucose concentration representing baseline value.

Serum biochemical analyses

Hematological Analysis

White blood cell (WBC), total and differentials, red blood cell (RBC), platelet counts, haematocrit, haemoglobin, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), were determined using an automatic blood analyser (URIT-3010 Automated Hematology Analyzer, Gullin, Guangxi, China).

Determination of serum markers in liver

Using commercial kits and following the manufacturer's instructions, the serum from each group was used to measure the levels of alanine aminotransferase (ALT), aspartate aminotransferase [AST], alkaline phosphatase [ALP], and total protein [Randox].

Determination of Renal parameters

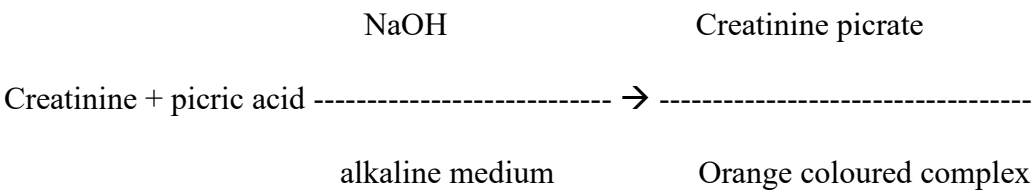
Electrolytes (Na+, K+ and Cl-) were determined using OPTITM LION Electrolyte Analyzer with Optical Fluorescence Technology [OPTI Medical Systems, Inc. Roswell, Georgia, USA]. The levels of urea [Randox, UK. Cat. No. UR 1068] and creatinine [CREA: Randox, UK. Cat. No. CR 510 and CR 524] in the serum were determined using commercial kits according to the manufacturer's direction.

Statistical analysis

The standard error of the mean (SEM) is the mean ± the data. To test for significant differences between the groups, one-way analysis of variance (ANOVA) was used. To determine if there was a significant difference between the means ($p < 0.05$), Duncan's multiple range test was employed.

SERUM CREATININE DETERMINATION

Serum Creatinine was determined using the alkaline picrate method (Jaffe's Method) (Toora et al., 2002) where creatinine reacts with picric acid in an alkaline medium to give an orange-coloured complex which shows maximum absorbance at 520 nm. The concentration of creatinine in the serum sample is correlated with the color's intensity and the results are expressed as mg/dl.



In brief, To obtain a clear supernatant, 1.5 ml of water, 1 ml of 10% sodium tungstate, and 1 ml of 2/3 N sulfuric acid were added to 0.5 ml of serum sample taken in a centrifuge tube. The mixture was then mixed and centrifuged at 2500 rpm for 10 minutes. This process precipitates the proteins from the sample. 1 ml of the supernatant was transferred into another

set of labelled test tubes containing equal quantity of (0.75 N) sodium hydroxide and 1.1% picric acid. Well combined, the mixture was allowed to sit at room temperature for fifteen minutes for the development of color. The intensity of the orange colour developed was measured at 520 nm using the Hitachi spectrophotometer against the reagent blank prepared by adding 3 ml of distilled water to 1 ml of sodium hydroxide and 1 ml of picric acid (Patlolla *et al*., 2019). standard curve prepared by taking known concentrations of creatinine solution ranging from 0.01-2 mg/dl and processed simultaneously in the same way as for the serum sample.

CHAPTER FOUR

4.0 RESULT

4.10 Clinical Observations.

Daily oral administration of aqueous fruit pulp extract from Picralima nitida at the tested doses (200, 500, 1000, 2000 and 3000 mg/kg body weight) for 35 days without causing the rats to exhibit any overt signs of toxicity or death..

Table 4.10
Mean comparison of electrolytes in the different treatment groups

	Control	200mg	500mg	1000mg	2000mg	3000mg	F	p
CREATININE	1.28±0.17[a]	1.34±0.07[a]	1.22±0.11[a]	1.22±0.09[a]	1.22±0.07	1.26±0.11[a]	0.190	0.963
UREA	30.96±5.06[a]	29.92±3.10	37.82±3.79[a]	35.32±2.70[a]	36.28±1.13	33.90±1.71[a]	0.931	0.479

The data are presented as mean±SEM (n = 5);
Means with distinct superscripts are statistically significant at P<0.05.

Comparing the treated groups to the control, there was no significant ($p > 0.05$) change in the levels of urea and creatinine. (Table 4.10)

Table 4.11
Mean comparison of Liver and Kidney Function Tests in the different treatment groups

	Control	200mg	500mg	1000mg	2000mg	3000mg	F	p
TOTAL PROTEIN	70.76±1.40[a]	66.52±1.09[a]	68.86±2.14[a]	69.68±2.94[a]	64.68±2.39[a]	66.86±1.97[a]	1.183	0.347
ALBUMIN	38.86±2.07[a]	36.94±3.91[a]	40.64±1.83[a]	37.14±3.45[a]	40.04±2.03[a]	40.28±1.20[a]	0.390	0.851

The values are presented as mean±SEM (n = 5);

P<0.05 indicates statistical significance for means with distinct superscripts.

When comparing the treated groups to the control, there was no discernible ($p < 0.05$) decrease in total protein or albumin. (Table 4.11).

CHAPTER FIVE

DISCUSSION

Renal dysfunction can be assessed by measurements of electrolytes, urea and creatinine (Akindele ct al., 2014). Waste products generated during the metabolism of proteins include creatinine and urea. After being transported to the kidney, both of these waste products are filtered into the urine. They are measured in order to assess the kidney's overall functioning. Renal failure is indicated by elevated urea and creatinine levels. Serum concentration of creatinine is constant under normal circumstances unless glomerular filtration rate (GFR) changes, as a result of defective renal function, and it is a good index of measuring GFR (Whitby et al. 1989).

The administration of aqueous fruit pulp extract from Picralima nitida resulted in a non-significant ($p > 0.05$) increase in creatinine levels, suggesting that renal function was not compromised during the study at the tested doses. Additionally, there was no discernible ($p > 0.05$) rise in urea level on administering the extract. However, plasma urea concentration is less reliable than creatinine as an index of GFR, because it diffuses into the renal tubular cells and its plasma concentration is dependent on the state of the liver function, protein intake and oxidation (Tilkian et al., 1979). The levels of electrolytes (sodium, potassium, chloride and bicarbonate) in blood are the outcome of fine regulatory mechanism of ionic charges and osmotic balance (Tilkian et al. 1979). The primary cation of extracellular fluid, sodium is controlled by the kidneys and adrenal glands. It is essential for maintaining the proper distribution of water and osmotic pressure in the different fluid compartments. An important marker of anion deficiency and electrolyte dispersion is plasma bicarbonate content. Acid-base imbalance is characterized by changes in bicarbonate and COz dissolved in plasma. This

imbalance can be brought on by renal tubular acidosis, hyperkalemic acidosis, renal failure, or keto acidosis. In this study, rats fed Picralima nitida aqueous fruit pulp extract showed a significant (ps0.05) increase in serum creatinine and urea when compared to the control group.

CONCLUSION

The study discovered that *P. nitida* aqueous fruit pulp extract are high in phytochemicals and have numerous medicinal and therapeutic importance which have been linked to a variety of biochemical and physiological effects. The usage of this plant extract particularly in determination and evaluation of Serum urea and creatinine values has provided widely accepted parameters to assess chronic kidney disease status as well as to assess renal status in susceptible diabetic and hypertensive subject. No wonder, the plant and even the fiber are widely utilized in ethno-medicine in West Africa to cure a variety of aliment. This study found out that the creatinine and urea are of great value in helping to ascertain the proper functioning of a normal kidney.

REFERENCE

Akanji, M. A., Salau, A. K., & Yakubu, M. T. (2013). Safety evaluation of aqueous extract of Crateva adansonii leaves on selected tissues of rats. *Fountain Journal of Natural and Applied Science*. 2(1), 17-28.

Burkill, H.M., (1985). The useful plants of West Tropical Africa. 2nd Edition. Volume 1, Families A–D. *Royal Botanic Gardens, Kew, Richmond*. 960 pp.

Corbett, A.D., Menzies, J.R.W., Macdonald, A., Paterson, S.J. & Duwiejua, M., (1996). The opioid activity of akuammine, akuammicine and akuammidine: alkaloids from Picralima nitida (fam. Apocynaceae). *British Journal of Pharmacology* 119: P334.

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. & Odelola, H.A., (2000). Evaluation of the antimicrobial property of the stem bark of Picralima nitida. *Phytotherapy Research* 14(5): 368–370.

François, G., Ake Assi, L., Holenz, J. & Bringmann, G., (1996). Constituents of Picralima nitida display pronounced inhibitory activities against asexual erythrocytic forms of Plasmodium falciparum in vitro. *Journal of Ethnopharmacology* 54: 113–117.

Iwu, M.M. & Klayman, D.L., 1992. Evaluation of the in vitro antimalarial activity of Picralima nitida extracts. *Journal of Ethnopharmacology* 36(2): 133–135.

Landry DW, Bazari H. Approach to the patient with renal disease. In: Goldman L, Schafer AI, eds.(2020). *Goldman-Cecil Medicine*. 106pp.

Menzies, J.R.W., Paterson, S.J., Duwiejua, M. & Corbett, A.D., (1998). Opioid activity of alkaloids extracted from Picralima nitida. *European Journal of Pharmacology* 350(1): 101–108.

Neuwinger, H.D., (1996). *African ethnobotany: poisons and drugs*. 941 pp.

Briefel G, Pincus MR. Evaluation of renal function, water, electrolytes, and acid-base balance. (2022) *Henry's Clinical Diagnosis and Management by Laboratory Methods*. 15:1-3

Omino, E.A., (1996). A contribution to the leaf anatomy and taxonomy of Apocynaceae in Africa. *Wageningen Agricultural University Papers*. 96–111, 178.

Park, M. Y., Choi, H. Y., Kim, J. D., Lee, H. S., & Ku, S. K. (2010). 28 Days repeated oral dose toxicity test of aqueous extracts of mahwangyounpae-tang, a polyherbal formula. *Food and Chemical Toxicology*. 48(8-9), : 2477-2482.

Ramirez, A. & García-Ribio, S., (2003). Current progress in the chemistry and pharmacology of akuammiline alkaloids. *Current Medicinal Chemistry* 10: 1891–1915

Toora BD; Rajagopal G Measurement of creatinine by Jaffe's reaction. (2002). *Journal of Experimental Biology. 40: 352–354.*