THE EFFECT AQUEOUS STEM BARK EXTRACT OF *Picralima nitida* ON THE INSULIN AND GLUCOSE LEVEL OF NORMAL SPRAGUE-DAWLEY RATS

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DEPARTMENT OF MEDICAL BIOCHEMISTRY

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UNIVERSITY OF BENIN

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IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN MEDICAL BIOCHEMISTRY

SUPERVISED BY

Dr. FIDELIS E. OLUMESE

MAY, 2024

TITLE PAGE

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A PROJECT WORK SUBMITED TO THE DEPARTMENT OF MEDICAL BIOCHEMISTRY, FACULTY OF BASIC MEDICAL SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCES (B.Sc) IN MEDICAL BIOCHEMISTRY

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CERTIFICATION

This is to certify that this project work was carried out by **OKORO DUMEBI ISABELLA** with matriculation number **BMS1902173**, of the Department of Medical Biochemistry, Faculty of Basic Medical Sciences, University of Benin, Benin city, in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc.) degree in Medical Biochemistry

Signed

....

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....

(EXTERNAL SUPERVISOR) (**Date)**

DEDICATION

I dedicate this to God Almighty my creator, my strong pillar, for his wisdom and direction during the course of this work.

I would also like to dedicate it to my parents, my siblings, my lovely course mates for their supports and also to my wonderful project supervisor, Dr. Fidelis E. Olumese for his guidance.

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First and foremost, my profound gratitude goes to God Almighty for his wisdom and direction throughout my research and write-up of this project work.

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To my mother, Mrs Theresa Okoro, I say a very big thank you for the constant care, prayer, sacrifices and support. To my Godmother (Dr. Mrs Gloria Mok), who has sacrificed a lot to see me through this academic journey, may God continue to bless you, I am forever grateful Ma, and to my sister (Miss Amaka Okoro) I say thank you for all your sacrifices and for loving me so dearly. I love you too and thanks for always been there for me. To Dr. Mrs Efedi, may God continue to bless you for your advice, care and support, I am very grateful Ma.

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ABSTRACT

Medicinal plants contain numerous phytochemicals that have the ability to prevent, manage, and cure various diseases. *Picralima nitida* has been used in herbal medicine since ancient times and possesses several pharmacological properties. This study aimed to access the toxicity effect of aqueous stem bark extract of *Picralima nitida* using healthy normal adult male Sprague-Dawley rats, investigate its effect on glucose and insulin secretion and analyze its effect on the pancreas. We carried out both acute and subchronic toxicity study. The acute toxicity study comprised of 15 rats and was carried out in 2 phases. At the end of acute toxicity test, there were no visible signs of toxicity observed in all the animals administered the extracts. Subchronic toxicity study comprised of 30 rats and lasted for over a period of four (4) weeks. Fasting blood sugar levels were measured in groups administered with doses of 150mg/kg, 300mg/kg, 800mg/kg, 2000mg/kg and 5000mg/kg. Across all groups, there was a consistent decrease in FBS when the baseline FBS is compared to the final week FBS. This suggests that the aqueous stem bark extract of *P.nitida* may be a potential hypoglycemic agent, although these changes are not statistically important. Insulin levels were measured to access the extract's impact on insulin secretion and compared to the normal control group (4.04±3.32), most doses did not significantly alter insulin secretion, except for the 150mg/kg dose which increased to about 4.30 ± 2.26^{a} and 5000mg/kg dose, which showed a substantial increase (27.94±23.21). However, this increase was not statistically significant ($p = 0.433$). The effects of the extract on the pancreatic weight showed a significant reduction across all doses compared to the normal control. Pancreatic weight decreased significantly at all administered doses (150mg/kg to 5000mg/kg) with $p = 0.000$, indicating a high level of statistical significance. The histology of the pancreas showed that there was no inflammation also the extract at 150mg/kg and 2000mg/kg dose increased Islets of Langerhans markedly. At 300mg/kg and 5000mg/kg there was no increase in Islets of Langerhans.

CHAPTER ONE

INTRODUCTION

1.0 Overview of medicinal plants

The use of plants in the treatment of ailments in Africa is an age long practice as nature has been a source of medicinal agents for thousands of years and a good number of modern drugs have been isolated from natural sources. Medicinal plants are used to maintain physical, mental and spiritual health in all cultures and in a variety of capacities and contexts (Davis and Choisy, 2024). Medicinal plants contain bioactive components that can be used for therapeutic purposes or that can serve as precursors for the manufacturing of beneficial drugs (Olumese *et al.,* 2023). Generally, these plants are sources of various phytochemicals, some of which are usually responsible for their biological effects and can be structurally optimized and processed into new drugs. These phytochemicals perform intermediary metabolic activities and they function both as primary metabolites, such as fats and sugars found in all plants and necessary for the plants life and secondary metabolites which are the remaining plant chemicals produced by the cells through metabolic pathways utilizing the primary metabolites (Bone and Mills, 2013; Hussein and El-Anssary, 2019). Medicinal plants therefore remain the most abundant natural primary source of active drugs and are invaluable in the ethnomedical treatment of diverse ailments (Ugboko *et al.,* 2020). Among the phytochemicals mentioned as potentially providing health benefits are polyphenols, flavonoids, anthocyanidins, alkaloids, phytoestrogens, terpenoids,

carotenoids, saponins, limonoids, phytosterols, glucosinolates among others (Teugwa *et al*.,2013).

1.1 Statement of problem

Picralima nitida, is believed to have anti-diabetic properties, yet scientific validations of this claim is scarce. This study aims to examine the effects of aqueous stem bark extract of *P. nitida* on insulin and glucose levels in Sprague-Dawley rats. By analyzing the impact of this extract on these key metabolic indicators, this study seeks to provide insights into its potential for managing diabetes and its related complications.

1.2 Justification of study

Picralima nitida is a therapeutic herb commonly called *Akuamma* or *Pile plant* belonging to the hunterieae tribe of the apocynaceae (Erharuyi *et al.,* 2014). This plant is used in ethnomedicine for the management of several disease conditions including diabetes (De-Campos *et al.,* 2020). It is widely used in Africa especially, Cameroon, Ghana and Nigeria. According to various research, the Stem bark, seed, fruit, leaf, from this plant is widely employed in the management of various disorders. However, this ameliorative effect of *P. nitida* may be attributable to the presence of specific compounds. Clinical investigation are now increasingly been done on the various parts of this plant to analyze the specific compounds necessary for the plants therapeutic effect and ascertain its toxicities.

Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells primarily myocytes and adipocytes {Tierney *et al.,* 2014). Diabetes management focuses on

maintaining blood glucose levels as close to normal as possible, while avoiding hypoglycemia and also maximizing insulin secretion. The importance of using herbal medicines to treat diabetes mellitus has grown worldwide. The World Health Organization has also recommended and supported this approach, especially in countries where conventional diabetes treatments are not readily available (WHO, 2000).

1.3 Objective of study

i. To access the in vivo glucose and insulin levels upon administration of stem bark extract of *Picralima nitida* in Sprague-Dawley rats.
ii. To investigate the toxicity levels of the aqueous stem bark extract of *Picralima nitida* through histopathological evaluation of the pancreas.

1.4 Aim of study

The main purpose of this research is to access the subchronic effect of aqueous stem bark extract of Picralima *nitida* using Sprague-Dawley rats with the goal of investigating its effect on glucose and insulin, making recommendations of its effective dose of administration and analyzing its toxicity levels using histopathology of the pancreas.

CHAPTER TWO

LITERATURE REVIEW

2.1 Distribution and local names of *Picralima nitida*

Picralima nitida is a shrub belonging to the Apocynaceae family, this plant is the first species of the genus *Picralima* to be described and is widely distributed in the vast majority of the tropical rain forests of equatorial Africa, notably in Cameroon, Nigeria, Congo, Ivory Coast, Democratic Republic of Congo, Ghana, and Uganda and its various parts are utilized for therapeutic purposes (Akabassi *et al.,* 2020; Akabassi *et al.,* 2017). In Nigeria, *P. nitida* is popularly referred to as *Osi-Igwe* by the Igbos*, Abere* by the Yorubas and *Osu* by the Edos. Elsewhere in West Africa, the plant is called *Gbe-Fondangné* (Benin Republic), *Adangme* (Ghana), *Abure ebissi* (Ivory Coast). *Picralima nitida* seed is commonly called *Akuamma* in Ghana (Obitte *et al.,* 2017).

P. nitida, demonstrates its adaptability, by expanding its presence into the Savannahs. Another captivating feature is its tendency to create riverbanks and water channels, finding comfort near flowing streams. Hence, the plant's distribution underscores its adaptability and resilience, showcasing its capacity to flourish in various environments across its native West Africa. This plant intertwines harmony, cultural importance, and biodiversity.

2.2 Description and classification of *Picralima nitida*

Picralima nitida (Apocynaceae) is an African pepper tree valued for its medicinal benefits (Olumese *et al.,* 2023). This plant reaches the height of 4-35 m, featuring a compact canopy and a trunk diameter ranging from 5-60 m. Its timber is cylindrical in shape and displays a pale

yellow hue (Okonta and Aguwa, 2007) .

Fig 2.1: *Picralima nitida* tree

Source: Wikipedia

The plant bears white flowers (about 3cm long) accompanied by ovoid fruits which at maturity are yellowish in colour. Its herbage showcases rectangular- shaped leaves between 6-20 cm in length and 3-10 cm in width and these leaves exhibit a robust nature and are characterized by the presence of 14-24 pairs of compact, delicate lateral veins (Burkill, 1985).

Taxonomical classification of *Picralima nitida*

KINGDOM	Plantae
DIVISION	Magnoliophyta
CLASS	Magnoliopsida
ORDER	Gentianales
FAMILY	Apocynaceae
GENUS	Picralima
SPECIE	Picralima nitida

Table 2.1: Classification of *Picralima nitida*

Source:wikipedia

2.3 Propagation of *Picralima nitida*

Picralima nitida plant can be grown through seed propagation or stem cutting propagation. Scarification, where the tough seed coat is intentionally damaged, is one technique used. Other methods involve cold treatments or soaking the seeds in water to replicate the natural conditions necessary for germination. Once prepared, the seeds are planted in nutrient-rich soil, either in well-maintained gardens or nurseries and as the seedlings develop, they are delicately

transplanted into larger containers and eventually into the fertile ground. Recently, there have been advancements in modern propagation techniques, leading researchers and scientists to investigate tissue culture. This method involves extracting small tissue samples from plants and cultivating them in controlled laboratory conditions (Schmelzer *et al.,* 2008).

2.4 Ethnomedical uses of *Picralima nitida*

Within the context of African ethnomedicine, numerous component of *Picralima nitida* including its leaves, fruits, and stem bark, have been employed for the remediation of different range of ailments including jaundice, malaria, abscesses, hepatitis, pneumonia, diabetes, hypertension and dysmenorrhea among others (Erharuyi *et al.,* 2014).The foliage serve as a vermifuge, while the extract derived from the leaves is applied directly to the ears to treat middle ear infections (Iwu *et al.,* 1992). In almost all of its distribution areas, the plant has high priority in the treatment of diseases such as malaria, diabetes, infectious diseases, tonsillitis, cancer, etc (Akabassi *et al.,* 2017).

2.4.1 Seeds:

The seeds of *Picralima nitida* contain essential minerals such as zinc, iron, and manganese, along with amino acids, and vitamins A and E (Adeola *et al.,* 2023).

In West Africa, particularly in Nigeria, cote d'ivore, and Ghana the seeds are widely used as an aphrodisiac (for sexual arousal), antipyretic, for the treatment of malaria, pneumonia, and chest

problems. A paste of the pulverised seed and shea butter is rubbed on the abdomen to treat leukorrhoea in women. In Gabon, the seeds are used topically to heal abscesses and this intense bitter seed of *P. nitida* can also be mixed with lemon juice for the treatment of diarrhoea and vomiting. In Ghana, the crushed seed is consumed orally for treatment of gastrointestinal disease, pneumonia, and chest complaint while the seed decoction is administered as an enema and analgesic.

Fig 2.2: *Picralima nitida* seeds

Source: Igweigbe *et al.,* 2021

2.4.2 Leaf

The leaves of *P. nitida* is a rich source of alkaloids, tannins, flavonoids, and saponins and has been used ethanomedically in the treatment of ulcers, sexual impotence; diabetes and hypertension (Bruce *et al.,* 2022).

In Cote d' Ivoire, Benin and Nigeria a leaf decoction is taken orally or used as a lotion (topical application) against measles. The dried leaves can be boiled in water and taken to treat guinea worms. In Sourthern Cameroon and Congo, the leaf sap is dipped into the ear to treat otitis (inflammation of the ear).

Fig 2.3: *Picralima nitida* leaf

Source: Wikipedia

2.4.3 Stem bark

The aqueous stem bark extract of *P.nitida* has a tremendous trypanocidal impact against Trypanosoma brucei, which was statistically comparable to that of diminazene aceturate (Berenil), commonly used in the treatment of sleeping sickness (resting disorders) (Wosu and Ibe, 1989).

In Nigeria, a decoction of the stem bark and root is taken as a bitter tonic and extensively used in place of quinine for the treatment of malaria and fevers. In Congo, a bark decoction is taken as a purgative, to cure cough, or taken with other plants to treat venereal diseases like gonorrhoea. An infusion of the bark is taken as a draught (either drunk or inhaled) in Ivory Coast, to treat yellow fever and jaundice. The bitter bark can also be boiled with sugar and the decoction is drunk against food poisoning. In Southern Cameroon and Congo, the stem bark decoction is drunk to cure sterility in male.

Fig 2.4: *Picralima nitida* stembark

Source: Yakeu and Serge, 2012.

2.4.4 Fruit

In Côte d'Ivoire, neglected vegetables and wild fruits such as those of *Picralima nitida*, which possess an abundance of vital bioactive elements like vitamins, minerals, and fibers, are utilized for both nourishment and in traditional remedies for ailments associated with oxidative stress, such as type-2 diabetes mellitus (T2DM) (Konan *et al.,* 2023). In Cameroon, the fruit decoction

is taken to treat cough or typhoid fever. In Ghana, the fruit shell is used to hold palm wine, which is then consumed after its bitter essence has been absorbed to relieve fever. The fruit is used to treat dysmenorrhea and digestive disorder in West Africa.

Fig 2.5: *Picralima nitida* fruit.

Source:Igweigbe *et al.,* 2021

2.5 Phytochemical composition of *Picralima nitida*

Phytochemicals (In Greek, "phyto" meaning "plant") are bioactive chemical compounds found in various parts of plant, including stems, leaves, roots, seeds, fruits, and flowers. These

phytochemicals exhibit antimicrobial, anti-diarrhea, anti-helmintic, anti-allergic, antispasmodic, and antiviral properties as well as strong antioxidant activity (Sharma *et al.*, 2018; Jaeger et al., 2016). In addition, they aid in controlling gene transcription, enhancing gap junction communication, boosting immunity, and offering defense against lung and prostate cancers [Rowles *et al.,* 2019; Jiang *et al.,* 2018; Cooperstone and Schwartz 2016; Vallverdo-Coll *et al.,* 2015; Yuan and Macquarrie 2015).

The remarkable abundance and diversity of phytochemicals within *Picralima nitida* highlights it as a valuable natural resource with immense potential for various applications such as pharmaceuticals, nutraceuticals, and cosmeceuticals. Studies conducted by various researchers including Teugwa *et al.,* (2013) consistently showed that extracts from are rich in various bioactive compounds. Their research unveiled a diverse range of phytochemicals in these extracts, such as polyphenols, flavonoids, anthocyanidins, alkaloids, phytoestrogens, terpenoids, carotenoids, saponins, limonoids, phytosterols, and glucosinolates, among others. Specifically, studies have highlighted that the seed extract of P. nitida contains glycosides, alkaloids, triterpenes, flavonoids, polyphenols, saponins, and tannins (Okonta and Aguwa, 2007; Olufunsho et *al.,* 2019; Teugwa *et al.,* 2013).

2.5.1 Alkaloids

Alkaloids are a class of organic compounds known for their analgesic pharmacological activity. Erharuyi *et al.,* (2014), reported that alkaloids are the major class of phytochemicals isolated

from *P. nitida* seeds. The first set of alkaloids isolated from *P. nitida* are the indole alkaloids and the names of these compounds were obtained from the indigenous name of the plant in Ghana 'Akuamma', they include akuammine, akuammidine, akuammicine, akuammigine and pseudo-akuammigine and are potent compounds which possess opioid analgesic activity (Obitte, *et al.,* 2017).Several alkaloids such as Picraphylline, picracine, picraline, picralicine, picratidine, picranitine, burnamine, pericalline and pericine have also been isolated from this plant. These alkaloids are known to exhibit therapeutic properties, such as analgesics, anti-inflammatory, and muscle-relaxing activity.

2.5.2 Flavonoids

Flavonoids are a significant group of natural compounds, specifically categorized as plant secondary metabolites with a polyphenolic structure, and are commonly present in fruits, vegetables, and some beverages (Panche *et al.,* 2016). This group of phytochemicals are abundant in *Picralima nitida* plant, these compounds gives the plants its vivid colors and also exhibits potent antioxidant properties that help protect against cellular damage caused by harmful free radicals. Flavonoids are linked to numerous health benefits and are crucial in many nutraceutical, pharmaceutical, medicinal, and cosmetic products. Their significance stems from their antioxidant, anti-diabetic, anti-inflammatory, anti-mutagenic, and anti-carcinogenic properties, along with their ability to influence key cellular enzyme activities. Additionally, they are effective inhibitors of various enzymes, including xanthine oxidase (XO), cyclo-oxygenase (COX), lipoxygenase, and phosphoinositide 3-kinase (walker *et al.,* 2000).

2.5.3 Saponins

Saponins are natural compounds known for their ability to foam when mixed with water, but their significance is greater than mere bubbles. Saponins are molecules with both hydrophilic and hydrophobic properties, composed of a carbohydrate part and either a triterpenoid or steroid aglycone. They are known for their diverse biological activities, including fungicidal, antimicrobial, antiviral, anti-inflammatory, anticancer, antioxidant, and immunomodulatory effects (Juang *et al.,* 2020).

2.5.4 Tannins

These are soluble astringent complex phenolic compounds that have been traditionally employed for their antimicrobial and antioxidant properties. Tannins are also believed to contain potential anti-inflammatory abilities and may contribute to wound healing (Praveen *et al.,* 2012). As astringent agent they are used as liquid cosmetics for cleansing the skin and contracting pores and also for checking the discharge of mucus or serum by causing shrinkage of tissue. Additionally, since the 18th century, they have been widely employed by leather manufacturers to enhance leather durability during the dyeing or tanning process. Tannins can precipitate gelatin on animal skins and produce a brownish color, which is why this group of phytochemicals are named as such (Falcão *et al.,* 2018). Tannins possess somewhat undesirable sensory characteristics, being bitter and imparting a brownish hue to foods. However, their exceptional antioxidant properties make them valuable as food additives to enhance shelf life and

safety, which has led to trials for their legal approval. Additionally, their ability to cause precipitation has made them useful as clarifying agents in the beverage industry for many years, particularly in beer, juices, and wines (Sharma *et al.,* 2019).

2.6 Pharmacological activities of *Picralima nitida*

The extracts from different parts of *Picralima nitida* have been found to possess a broad range of pharmacological activities which lends credence to its ethnomedicinal uses.

2.6.1 Antidiabetic effects

Given the widespread occurrence of type 2 diabetes in Africa, it's important to explore sustainable and healthier alternatives that are not only more affordable but also entail fewer side effects compared to costly conventional medical interventions (Konan *et al.,* 2023).

Picralima nitida is used in traditional medicine for the treatment and management of malaria, abscesses, hepatitis, pneumonia, diabetes, and hypertension (Erharuyi *et al.,* 2014; De-Campos *et al.,* 2020; Teugwa *et al.,* 2013). With the aid of a local grinder the seeds are pulverized and added to foods such as akamu (known as pap in English) (Shittu *et al.,* 2010) or taken as a decoction. The study by (De-Campos *et al.,* 2020), examined the potential palliative effect of aqueous seed extract of *Picralima nitida* (APN) on dyslipidemia, hyperglycemia, oxidative stress, insulin resistance, and the expression of some metabolic genes in high-fat high-fructose-fed rats. Teugwa *et al.,* (2013), investigated the antidiabetic potential of methanol and hydroethanol

extracts of the stem bark and leaves of *P. nitida* in streptozotocin-induced diabetes in mice. The result of the study showed that the methanol leaf extract of *P. nitida* at 300 mg/kg exhibited significant antidiabetic activity with 38.48% blood glucose reduction.

2.6.2 Analgesic activity

Analgesics are substances that help alleviate pain and discomfort, offering relief to those in need. In the past, local communities have utilized this plant for its diverse medicinal properties including its remarkable analgesic effects.

Scientific studies has demonstrated that extracts derived from *Picralima nitida* plants possess significant analgesic properties, these extracts effectively interact with the body pain perception pathways, helping to alleviate pain and improve overall well-being.*P. nitida* seeds contain a mixture of alkaloids producing antipyretic and anti-inflammatory effects along with analgesia in animal studies (Duwiejua *et al.,* 2002). The alkaloids in the seeds interact with opioid receptors in the brain leading to its analgesic effect similar to opioids but with milder side effects. The assessment of the analgesic effects of ethanol seed extract from *P. nitida* revealed a dose-dependent increase in the average pain threshold of rats. Additionally, it notably inhibited bradykinin-induced hyperalgesia in rats (Ezeamuzie *et al.,* 1994). Alkaloid has been found to be three (3) times stronger than cocaine hydrochloride (Bravo *et al.,* 2022). In Ghana and Cameroon, a decoction of the seed is used as a painkiller for chest pain and acute stomach problems.

2.6.3 Antimicrobial activities

Picralima nitida exhibits remarkable antimicrobial effects. Its stem bark, roots and leaves is a rich reservoir of natural defenses, harnessing their power to combat a diverse range of pathogens. Scientific investigations have illustrated the antimicrobial prowess of the extract derived from the stem, roots, and leaves of the plant.

Ethanol, benzene, chloroform and aqueous (cold and hot) extracts of *P. nitida* (seed, stem bark, leaves and root) were tested against five strains of bacterial namely*: Escherichia coli, Psuedomonas aeruginosa, Bacillus subtilis, Staphylococcus aureus* and *Salmonella kintambo* using the agar-well diffusion method. The ethanol extracts from both the root and stem bark showed activity against all test organisms, while the benzene and chloroform extracts displayed no activity (Nkere and Iroegbu, 2005). The antimicrobial effect of *P. nitida* extends to fungal strains, further amplifying the plants potential in addressing fungal infections. . The chloroform extract of the seed of P. nitida was evaluated for possible anti-leishmanial activity using a radiorespirometric micro-test technique and the result of this study confirmed its activity against Leishmania donovani at 50 µg/ml dose (Iwu *et al.,* 1992). These antimicrobial effects of *Picralima nitida* can be attributed to diverse array of bioactive compounds, including alkaloids, saponins and flavonoids, which work synergistically to disrupt the growth and proliferation ofmicroorganism (Anand *et al.,* 2019). These compound basically acts as natural warriors, targets the cellular structures of pathogens and preventing their ability to cause harm.

2.6.4 Antimalarial properties

Picralima nitida, has traditionally been used to treat malaria, the bark, root, leaves, fruits and seeds of the tree has anti-malarial properties.

The alkaloid picraline has been proven to stop the growth of the parasite that causes malaria (Plasmodium falciparum) by interfering with its hemoglobin digestion process, a process critical for the parasites survival. These alkaloid extracted from the plant exhibit activity against drug-resistant and drug sensitive malaria strains of Plasmodium falciparum and also show significant inhibitory activity against both clones of P. falciparum (Iwu and Klayman, 2002). Additionally, the alkaloids may also interfere with the parasite ability to invade red blood cells, further limiting its ability to cause diseases. Some indole alkaloids has been isolated from the seeds and have shown activity against the chloroquine-resistant type of Plasmodium falciparum.

2.6.5 Antipyretic activity.

Antipyretics are substances that reduce fever. They work by lowering an elevated body temperature, which is often a symptom of an underlying condition such as an infection or inflammation.

In West African folk medicine, *P. nitida* is widely used for the treatment of several diseases and also as a febrifuge (Akabassi *et al.,* 2017). The antipyretic activity exhibited by P. nitida could be due to the presence of unique bioactive compounds, including alkaloids and flavonoids

(Duwiejua *et al.,* 2002). In Ghana, the fruit shell is used to hold palm wine, which is then consumed after its bitter essence has been absorbed to relieve fever. Studies have shown that extracts from this plant possess antipyretic effect as these extracts effectively modulate the body temperature-regulating mechanisms, helping to lower fever and restore the body to a state of balance.

2.6.6 Antioxidant properties

Antioxidants are substances that can significantly delay or completely prevent the oxidation of substrate molecules, even at low concentrations. Antioxidants are the first line of defense against free radical damage, and are therefore important for maintaining optimum health and well being.

An imbalance between free radicals and antioxidants leads to oxidative stress which in turn results in the development of pathological diseases, one of which is diabetes. Oxygen is necessary for aerobic life, but, under certain conditions such as when toxic levels has been generated leading to cellular injury it is responsible for causing a variety of diseases including Alzheimer's disease, Parkinson's disease, early onset of aging, cancer, neuronal disorders, and cardiovascular disease (Ames, 1983; Leong and Shui, 2002;Li *et al.,* 2007; Lopes *et al.,* 1998).The study by Asmat *et al.,* (2016), have demonstrated that oxidative stress due to lipid peroxidation, led to a reduction in vitamin C levels, impaired glutathione metabolism, and also brought changes in enzymatic systems and all this are linked to the pathogenesis of diabetes. The seeds of *Picralima nitida* have been shown to be rich in amino acids, vitamins A and E, as well

as minerals such as zinc, iron and manganese (Adeola *et al.,* 2023), which works synergestically with antioxidants to prevent cellular damage.

2.6.7 Antiulcer properties

P. nitida seed has been found to reduce or eliminate the formation of ulcers which are sores or breaks in the lining of the gastrointestinal tracts.

In a study by (Okonta *et al.,*2011), they demonstrated the antiulcer activity ofseed extracts *P. nitida* on rats by oral administration of the methanol extract, chloroform fraction and methanol fraction at 1000 mg/kg body weight and this reduced gastric ulcer by 56.4%, 40.0%, and 56.3%, respectively.

2.7 Overview of the Pancreas

The pancreas (which means all flesh), is an elongated, tapered organ located in the upper abdomen behind the stomach (Longnecker, 2014). The widest part of the organ is the right side, called the head and it lies in the curve of the duodenum (first division of the small intestine). The tapered left side called the tail, extends slightly upward to the body of the pancreas and ends near the spleen (Vikash *et al.,* 2019). This organ is a part of the gastrointestinal system as it makes and secretes digestive enzymes into the intestine (exocrine pancreas), and also an endocrine organ that makes and secretes hormones into the blood to control energy metabolism and storage throughout the body (Longnecker, 2014). The enzymes secreted by the exocrine gland in the

pancreas help break down carbohydrates, fats, proteins, and acids in the duodenum. These enzymes travel down the pancreatic duct into the bile duct in an inactive form and they are activated when they enter the duodenum. The exocrine tissue also secretes bicarbonate, to neutralize stomach acid in the duodenum. The endocrine gland in the pancreas mainly secrete hormones such are insulin and glucagon, which regulate the level of glucose in the blood and somatostatin which regulate the release of insulin and glucagon. Therefore, the pancreas is a part of the digestive system and produces insulin and other important enzymes and hormones that help break down food (Vikash *et al.,* 2019). The exocrine components includes acinar and duct cells with associated connective tissue, vessels, and nerves and comprise more than 95% of the pancreatic mass while the endocrine component made up of the islets of Langerhans comprise 1-2% of pancreatic mass (Longnecker, 2014).

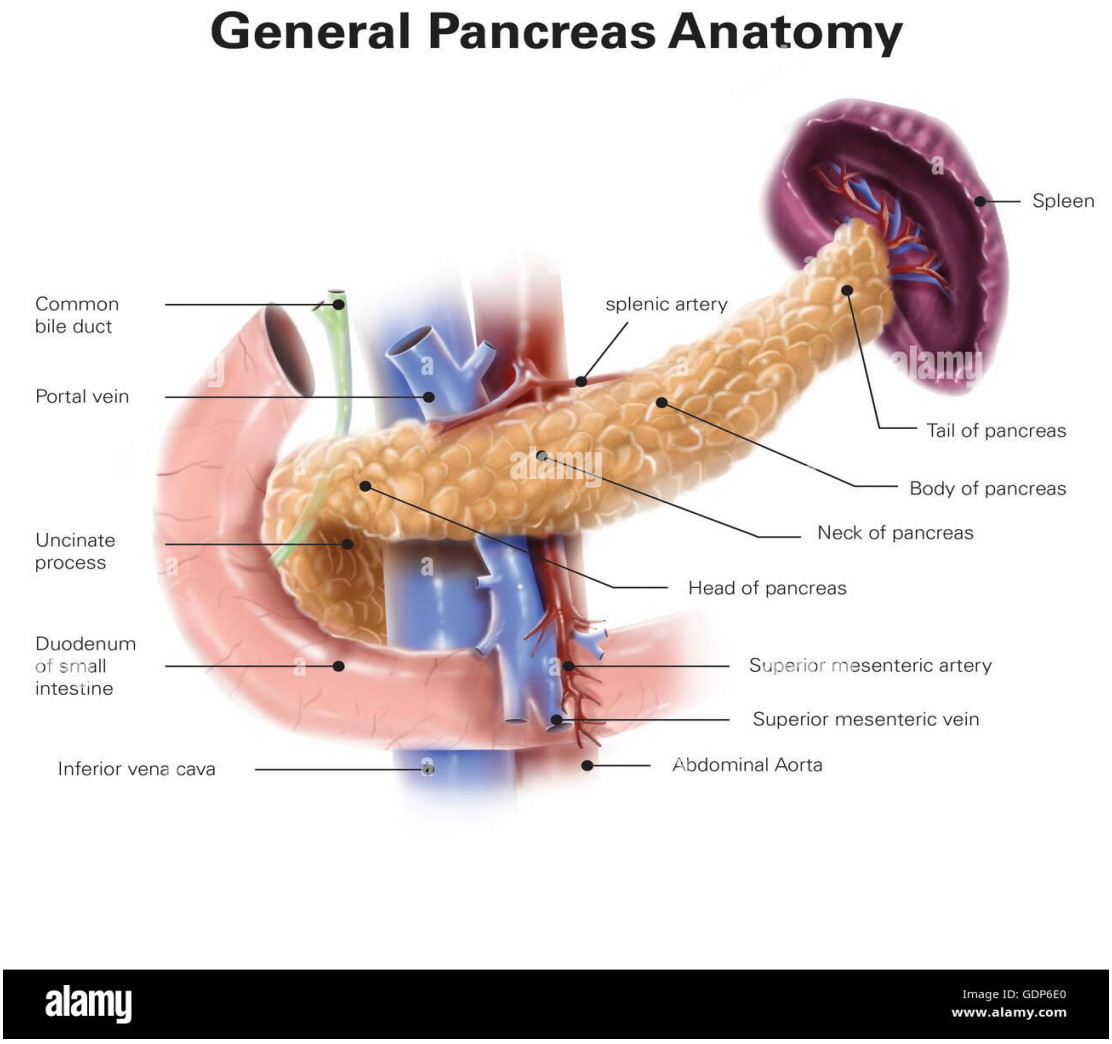

Fig 2.6: The general pancreas anatomy

Source: www.alamy.com

Fig 2.6: shows the gross anatomy of the pancreas and its relationship to surrounding organs in adults. Various portions of the pancreas are usually referred to as head, body, and tail. The head lies near the duodenum and the tail extends to the hilum of the spleen (Longnecker, 2014).

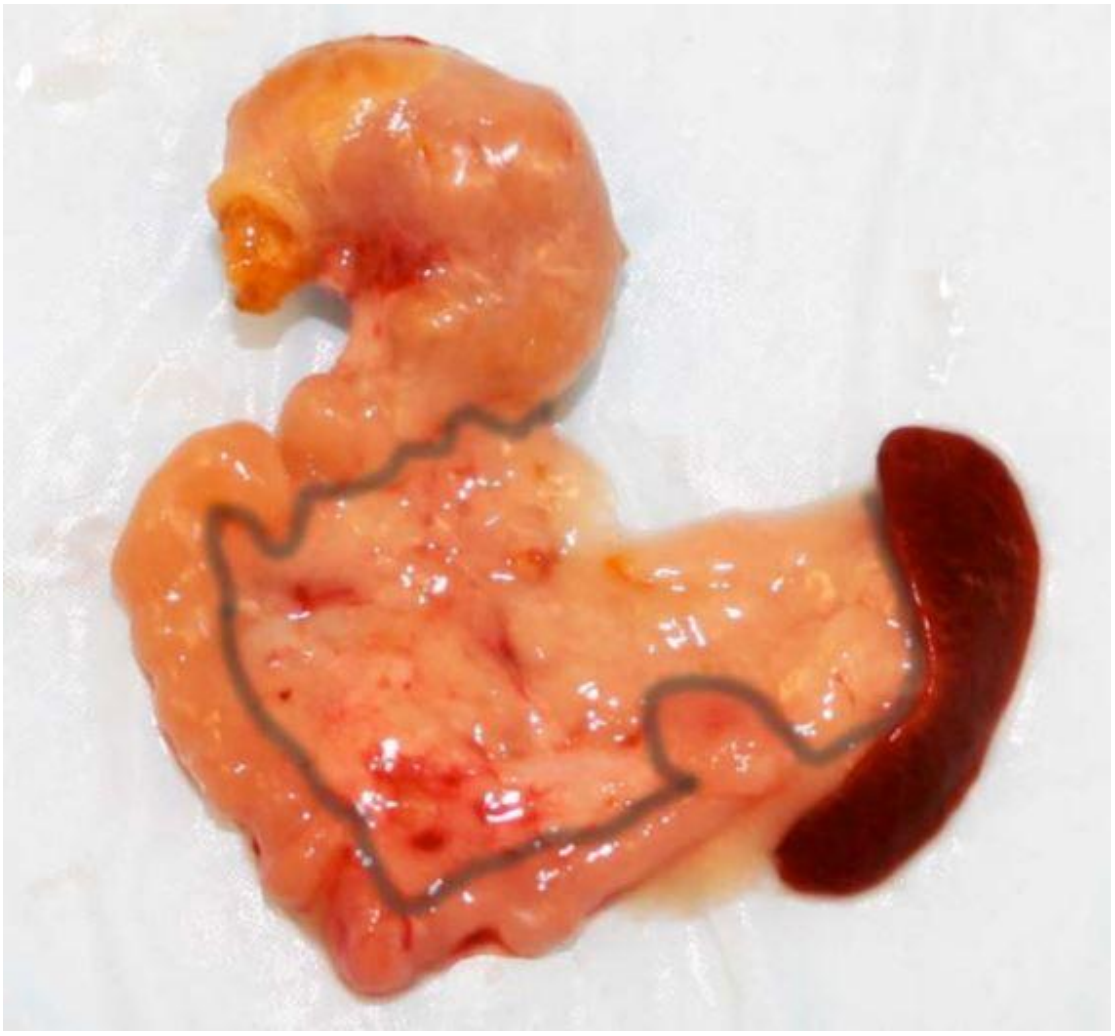

Fig 2.7: Mouse pancreas

Source: Longnecker, 2014.

Fig 2.7: depicts the pancreas of an adult mouse which is surrounded by the stomach (top), the duodenum and proximal jejunum (image left and bottom), and the spleen (image right). The duodenum invests the head of the pancreas (as demarcated by the line). Mouse pancreas is soft and broad (extended) compared with the human pancreas (Longnecker, 2014).

2.8 Glucose a fundamental molecule

Glucose (a simple sugar) is a fundamental molecule that serves as a primary source of energy for living organisms. It plays a crucial role in cellular respiration, providing fuel for metabolic processes. Additionally, glucose serves as a building block for larger carbohydrates like starch and cellulose and is crucial for the functioning of various tissues and organs in the human body, including the brain, muscles, and red blood cells. Eguchi *et al.,* (2018), highlights the traditional understanding of glucose homeostasis, which primarily focuses on the role of insulin in controlling blood glucose levels. Insulin promotes glucose uptake by cells, inhibits hepatic glucose production, and facilitates glucose storage as glycogen. Dysregulation of insulin signaling is a hallmark of conditions like type 2 diabetes.

2.9 Insulin

Insulin is a hormone produced by the beta cells of pancreas located in the Islet of Langerhans and it enables the cells of the body to absorb glucose for metabolic process. Insulin receptors are widely distributed throughout the body but the main targets are liver, fat, and skeletal muscle cells (in fat and skeletal muscle, insulin greatly up-regulates Glut4).However, when the cells of the body fail to take in glucose, it accumulates in the blood and results in many abnormalities (Busari *et al.,* 2015). Insulin resistance (IR) is a pathophysiological condition that refers to decreased sensitivity and responsiveness to insulin by its target organs. IR disrupts glucose uptake in muscle while promoting gluconeogenesis in the liver. Epidemiological studies have

shown that IR can pose a significant threat to health. It could lead to dyslipidemia, obesity, hypertension, diabetes, and cardio and cerebrovascular diseases. This chronic metabolic disorder often appears in the early stage of the disease and, if not detected or treated, can lead to type 2 diabetes and metabolic syndromes. Several studies have found that IR may be present several years before the diagnosis of type 2 diabetes (Laakso, 2015).

2.9.1 Role of insulin in management of diabetes

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia caused by insufficient insulin secretion or insulin resistance (IR). IR is the primary etiology of type II diabetes mellitus, accounting for over 90% of diabetic cases (Folorunso *et al.,* 2022). It is marked by chronic hyperglycaemia and alteration of carbohydrate, proteins and lipids metabolism associated with abnormal secretion and/or activity of insulin (Valiathan, 1998). When insulin signaling is defective, the insulin receptor substrate (IRS) phosphorylation and the PI3K/Akt pathway are inhibited which damages the AMPK phosphorylation and GLUT4 translocation to the cellular membrane, contributing to decreased glucose uptake in the skeletal muscle and adipose tissue (Huang, et al., 2018). In the liver, the defective PI3K/Akt pathway promotes gluconeogenesis by increasing the expression of phosphoenol pyruvate carboxykinase (PEPCK) and glucose 6-phosphate (G6P) while inhibiting glycogen synthesis by suppressing glucokinase (GK) and glycogen synthesis kinase (GSK).

2.9.2 *Picralima nitida*: a hope for diabetes management

A primary therapeutic target for the management of type 2 diabetes is the use of alpha-glucosidase and alpha-amylase enzyme inhibitors to slow the absorption of sugars ingested by reducing the uptake of glucose from the intestine (Eluehike *et al.,* 2023). Assumptions are that the sudden rise in blood glucose levels after a meal can be efficiently managed by inhibiting these enzymes to prevent them from hydrolyzing carbohydrates.

Numerous experimental studies have revealed that flavonoids can greatly improve insulin sensitivity via glucose and insulin associated signaling pathways. In comparison with synthetic pharmaceutical drugs, herbal medicinal plants have continued to show increasing prospects in the management of diabetes mellitus as they have been shown to have outstanding efficacy and have fewer side effects. *Picralima nitida* is one such medicinal plant that has been reported previously to show profound anti-diabetic effects using extracts from the seeds, stembarks, and leaves (Erharuyi *et al.,* 2014). Herbs are alternatives to synthetic drugs for diabetes treatment due to their perceived acceptability, effectiveness, safety, low cost, and fewer side effects in clinical experience (Aditya *et al.,* 2012).In spite of these interesting pharmacological reports, several aspects of the anti-diabetic mechanism of *P. nitida* still remain elusive. Furthermore, the potential of *P. nitida* in alleviating the oxidative stress-induced complications of diabetes mellitus remains unknown. For the treatment of type II diabetes mellitus, the ideal anti-diabetic medication would not only normalize blood glucose levels but also modulate critical pathways involved in insulin sensitivity.

CHAPTER THREE

MATERIALS AND METHOD

3.0 Collection and identification of plant

Plant name: Picralima nitida

Family: Apocynaceae

Common names: Picralima, Pile plant, Akuamma

Local names: Osu (Edo), Abere, Erin, Agege, Agege-arin (Yoruba), Osi-igwe (Igbo)

Stem bark of *Picralima nitida* was obtained locally at Oyingbo market in Lagos State, Nigeria, it was then transported to the department of Medical Biochemistry, University of Benin, Nigeria. A sample of it was taken to the Department of Plant Biology and Biotechnology, University of Benin, where it was identified, authenticated and given herbarium specimen (voucher Number) UBH-P424.

3.1 Materials / apparatus used for the experiment include:

- Grinder
- Transparent bowl
- Cotton wool

- Cheese cloth
- Handkerchief
- Plastic bottle
- Gloves
- Rubber band
- Keg(gallon)
- Electronic sensitive weighing balance
- Funnel
- Glass beaker
- Glass stirrer
- Spectrophotometer
- Measuring cylinder
- Laboratory coat
- Bin bags
- Desiccator
- Refrigerator

Reagents used for the experiment includes:

- Distilled water
- Chloroform
- 10% buffered formalin

3.2 Extraction Procedures

The obtained stem bark of *Picralima nitida* was thoroughly rinsed underneath running tap water to remove impurities. Afterwards, it was broken into pieces to help it dry faster, the broken stem bark was then dried in the laboratory at room temperature to preserve its phytochemical constituents because heat drying or drying directly under the sun can destroy its phytochemical compounds, It was allowed to dry for a 14-day period (2 weeks) and then ground into fine powder at Uselu market using a grinding machine. After pulverization, the plant was weighed with an electronic sensitive weighing balance and the weight obtained was 4.8kg. Exhaustive extraction process was used, the goal of exhaustive extraction is to achieve maximum extraction efficacy and to realize enough phytochemicals.

Aqueous extraction: The pulverized plant was poured into a transparent bowl and enough distilled water was added to completely cover it (until the distance between solute and solvent was 2cm), the mixture was allowed to stand for 72 hours with consistent vigorous stirring using a glass stirrer. Throughout the extraction process the transparent bowl was left open to prevent the growth of mould (as this usually occurs with aqueous extraction). Exhaustive extraction was carried out 3 times.

- First extraction: 3 days(72 hours)
- Second extraction: 2 days(48 hours)
- Third extraction: 1 day(24 hours)

To ensure that the extraction was effective, the solution was frequently stirred every morning and evening to;

- allow for the homogenization of mixable liquids and increase the speed of chemical reaction
- balance the differences in temperature
- prevent mucus and bacteria growth

Upon the conclusion of the third day, the solution went through a careful filtration process, it was passed through a bi-layered cheese cloth once, then densely packed cotton wool was layered on the cheese cloth (to trap the residues) and the decantation process was repeated 3 times (that is, a total of 4 times) till there was no residue present. After the exhaustive extraction, the final extract was put into a keg or gallon (with the aid of a funnel and handkerchief) then taken for freeze-drying. The freeze-dried extract was obtained in powdery form and conserved inside a refrigerator until it was time for use.

3.3 Ethical approval

For this study, fifteen (15) adult male Sprague-Dawley rats will be used for the acute toxicity test and thirty (30) adult male Sprague-Dawley rats for the subchronic toxicity test. The animals were to be purchased from the animal experimental unit of the department of Anatomy, faculty of basic medical sciences, University of Benin, Nigeria and would be transported to the department of Medical Biochemistry, University of Benin. They were to be housed in clean plastic cages with wood chip bedding, under natural day/night cycle, at ambient temperature. For each study

the animals would be acclimatized for two (2) weeks prior to experimental regimen. The animals would be housed, fed and cared for in accordance with the guidelines of Research Ethics Committee of the College of Medical Sciences, University of Benin. This research was approved by the Research Ethics Committee of the College of Medical Sciences, University of Benin.

3.4 Experimental design

In toxicology studies, experimental designs are tailored to evaluate the safety and potential risks associated with exposure to chemicals, drugs or other substances. Among the most common designs are acute and subchronic toxicity tests, which differ in terms of duration, dosing regimen and objectives.

3.4.1 Acute toxicity test

The acute toxicity test was carried out to evaluate the effect of a single dose of aqueous stem bark extract of *Picralima nitida* on the experimental rats. This test helps determine the lethal dose (such as LD50 which is the dose at which 50% of the test population dies). This test also helps to determine the toxic effects that might occur with acute (short term) exposure.

A total of fifteen (15) rats were used for the acute toxicity test. After acclimatization to animal house conditions for two weeks with free access to pellet feed and water, the rats were randomized into two phases.

- **Phase 1**

In phase 1, a total of twelve (12) rats were used and these rats were assigned four (4) groups of three (3) rats each. Group I, was the control and they were only given food and water while the other groups (II, III, IV) were given 10mg, 100mg and 1000mg of the extract per kg body weight respectively. Rats in groups II, III, IV are the test rats. Experimental rats in each group were assigned alphabetical labeling a, b, c (that is, rat a, rat b and rat c). The animals were dosed once, the route of administration was oral through a gavage and then the animals were observed for a period of two (2) weeks to check for clinical signs. Throughout the observation period the animals were given food and water.

- **Phase 2**

In phase 2, a total of four (4) rats were used and these rats were assigned into four (4) groups.
Group I, comprised of one (1) of the same control rat used in phase 1 and it was only given food and water while the other groups (II, III IV) were the test rats and they comprised of one (1) rat each. The rats in group II, III and IV were given 1600mg, 2900mg and 5000mg of the extract per kg body weight respectively. The experimental rats were labeled a, b and c.
The animals were dosed once, the route of administration was oral through a gavage and then the animals were observed for a period of two (2) weeks to check for clinical signs. Throughout the observation period the animals were given feed and water.

Animal sacrifice

Upon culmination of the acute toxicity test (i.e after phases 1 and 2), the animals were sacrificed.

3.4.2 Subchronic toxicity test

Subchronic toxicity test was carried out to evaluate the effect of repeated exposure of the aqueous stem bark extract of *Picralima nitida* on the experimental rats over a period of twenty-eight (28) days. This test plays a critical role in identifying the potential chronic toxic effects of the extract, determining safe exposure levels, establishing No Observed Adverse Effect Levels (NOAELs) and guiding further research or regulatory decisions.

A total of thirty (30) rats were used for subchronic toxicity test. The rats were acclimatized to animal house conditions for two weeks with free access to pellet feed and water, their body weight as well as their fasting blood glucose (FBG) level was measured at the end of the week. This initial FBG is called baseline glucose and prior to obtaining the baseline glucose the experimental animals were made to fast overnight (for 12 hours).

The rats were randomized into six (6) groups of five (5) rats each. Rats in group I were the control rats and for they were only given pellet feed and water throughout the experiment. Rats in the other groups were the test rats and rats in each group received the following standard dose of extract:

- Group II - 150mg/kg body weight
- Group III - 300mg/kg body weight
- Group IV - 800mg/kg body weight

- Group V - 2000mg/kg body weight
- Group VI - 5000mg/kg body weight

The rats were kept in six (6) different cages and rats in each cage were marked (using ink) on various parts of their bodies and tagged head, leg, hand, tail rats, for rats 1, 2, 3, 4 respectively while rat 5 was not marked and was tagged plain rat. The cages were cleaned weekly to preserve the health of the rats and prevent microbial infections. Throughout the testing period, the rats were weighed weekly, to prepare a stock solution of the extract and they were dosed daily (that is, weight of each rat was taken every week and a fresh stock solution was prepared while this extract was administered to the rats daily).

3.5 Fasting blood glucose estimation methodology

Fasting blood glucose (FBG) estimation is a critical diagnostic tool for assessing glucose metabolism in the body. It serves as an essential part of a comprehensive approach to managing blood glucose levels and assessing overall health. It also plays a crucial role in diagnosing, monitoring and managing diabetes mellitus. Other applications are the detection of neonatal hypoglycemia, the exclusion of pancreatic islet cell carcinoma as well as the evaluation of carbohydrate metabolism in various diseases.

During the subchronic toxicity test which lasted for 28 days, the experimental animals were made to fast overnight for 12 hours, a lancet was used to puncture their tail tip and the fasting blood glucose level was measured using a finn test strip and glucometer. Therefore, asides the

baseline reading (which is the initial FBG level gotten at the end of the acclimatization week), four (4) other glucose reading was taken every 7 days. The baseline fasting blood glucose obtained was.

3.6 Insulin assay methodology

Insulin is a crucial hormone that regulates blood sugar levels and it plays a pivotal role in metabolic health. Insulin test, typically referred to as a fasting insulin test or insulin level test, is conducted to measure the amount of insulin in a person's blood. Our aim was to assess insulin sensitivity, of the test rats after administration of the aqueous stem bark extract of *Picralima nitida*.

Clinical significance

Insulin is an anabolic hormone, whose main role is to promote the oxidation of glucose and synthesis of glycogen, and also to prevent the breakdown of glycogen so as to maintain blood sugar levels. In insulin deficiency or resistance, the blood glucose concentration increases, this increase can exceed the renal sugar threshold, leading to a wide range of metabolic syndrome and an occurrence of insulin-dependent diabetes.

Kit Used

Detection kit for insulin (INS)- Enzyme-linked immunoSorbent assay (ELISA). It is used in quantitative test for insulin in human serum.

Principle

INS kit uses a “Sandwich-ELISA principle” Enzyme linked immunological sorbent assay. To measure INS levels in serum, plastic wells coated with a monoclonal antibody of INS are supplied in the kit. After the patients specimen and another mono-antibody labeled with HRP are added, INS, is fixed to the solid phase antibody and creating a HRP antibody-INS-antibody “sandwich”. After incubation the unbound conjugate is washed off. The amount of the bound peroxidase conjugate is proportional to the concentration of the patient sample.

Assay Procedure

1) Mark the microtitration strips to be used. All the calibrators and control should set duplicate.
2) Dispense 50 µl of calibrators as controls/samples into wells.
3) Dispense 50 µl of HRP conjugate to each well.
4) Cover the strips with a plate sealer. Mix it gently by swirling the microtitre plate on flat bench. Incubate the plate at 37 degrees centigrate for 60 minutes.
5) Wash each well 3 times, 10 seconds each time.
6) Dispense 50 µl of chromogen A to each well.
7) Dispense 50 µl of chromogen B to each well.
8) Cover the strips with a plate sealer. Mix it gently by swirling the microtitre plate on flat bench. Incubate the plate at 37 degrees centigrate for 15 minutes.
9) Dispense 50 µl of stopping solution to each well and mix completely.
10) Read the absorbance of the plate within 10 minutes.

3.7 Histological analysis methodology

The dissected organs were fixed in 10 percent formalin saline. Fixed tissues were completely dehydrated in ascending concentrations of alcohol (70, 90, 96 and 100 percent). The tissues were placed in xylene to remove the alcohol, impregnated and embedded with molten paraffin wax. They were allowed to solidify before sectioning into 4um using a micro-tome (Leica RM 2235, UK) the 4um sections were placed on slides and stained with hematoxylin- eosin dye (Bancroft and Gamble, 2006). Stained slides were viewed using an optical photomicroscope (Olympus 230 V 50/60 He, Germany) and camera (Eakins 12Mega pixels, UK) x100 magnification

3.8 Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). Values obtained were examined using one-way analysis of variance (ANOVA), followed by a post-hoc test, to determine significant differences among groups. IBM SPSS software version 22 was used to analyse all data. Values were considered statistically significant at $P < 0.05$.

Animal sacrifice and sample collection

At the end of the 28 days administration, the rats were weighed using a weighing balance, made unconscious using chloroform and then sacrificed to obtain the pancreas. The collected pancreas was put in a sample bottle, preserved with formalin and sent to the laboratory for biochemical analysis and histological processing.

CHAPTER FOUR

RESULTS/FINDINGS

4.1 Acute toxicity test

No visible toxicity sign was observed.

4.2 Subchronic toxicity test

The statistical analysis results and the histological results gotten after the subchronic toxicity test include:

4.2.1 Effect of different doses of aqueous stem bark extract of Picralima nitida on fasting blood sugar (FBS) levels.

	Normal control	**150mg/kg**	**300mg/kg**	**800mg/kg**	**2000mg/kg**	**5000mg/kg**	**F**	**P**
Baseline FBS(mg/dl)	90.20±4.88[a]	83.40±2.54[a]	76.40±5.99[a]	90.20±3.71[a]	90.00±7.31[a]	96.00±6.78[a]	1.558	0.210
FBS after 1 week (mg/dl)	81.40±7.78[a]	97.60±10.65[a]	69.80±4.65[a]	76.20±3.18[a]	90.80±6.21[a]	73.20±4.68[a]	2.614	0.050
FBS after 2 week (mg/dl)	78.80±12.35[a]	81.20±4.60[a]	68.20±3.12[a]	78.20±8.43[a]	101.00±13.63[a]	80.60±8.57[a]	1.345	0.280
FBS after 3 week (mg/dl)	79.80±1.46[a]	86.00±6.80[a]	72.00±5.34[a]	65.60±7.89[a]	69.00±6.27[a]	63.60±3.87[a]	2.324	0.074
FBS after 4 week (mg/dl)	73.40±3.67[a]	71.20±3.37[a]	72.80±7.77[a]	73.80±2.40[a]	67.80±1.74[a]	73.80±1.46[a]	0.340	0.883

Values are expressed as mean±standard error of mean. Means with different superscripts are statistically significant at $p<0.05$.

There was no significant variation in the fasting blood glucose levels of the animals from all groups administered different doses (150mg, 300mg, 800mg, 2000mg and 5000mg per kilogram body weight) of aqueous stem bark extracts of *p. nitida* compared to the control.

4.2.2 Effect of different doses of aqueous stem bark extract of Picralima nitida on insulin secretion

	Normal control	150mg/kg	300mg/kg	800mg/kg	2000mg/kg	5000mg/kg	F	P
Insulin	4.04±3.32[a]	4.30±2.26[a]	4.02±0.87[a]	4.07±0.43[a]	3.75±1.67[a]	27.94±23.21[a]	1.023	0.433

Values are expressed as mean±standard error of mean. Means with different superscripts are statistically significant at p<0.05

There was no significant variation in insulin secretion of the animals from all groups administered different doses (150mg, 300mg, 800mg, 2000mg and 5000mg per kilogram body weight) of aqueous stem bark extracts of *p. nitida* compared to the control.

4.2.3 Effect of different doses of aqueous stem bark extracts of Picralima nitida pancreatic weight

	Normal control	150mg/kg	300mg/kg	800mg/kg	2000mg/kg	5000mg/kg	F	P
Pancreas	0.54±0.02[a]	0.34±0.02[b]	0.32±0.02[b]	0.34±0.02[b]	0.34±0.02[b]	0.36±0.02[b]	12.047	0.000

Values are expressed as mean±standard error of mean. Means with different superscripts are statistically significant at p<0.05

There was a significant difference in the weight of the pancreas (F = 12.027, P = 0 .000) in all groups administered different doses (150mg, 300mg, 800mg, 2000mg and 5000mg per kilogram body weight) of aqueous stem bark extracts of *P. nitida* in comparison to the normal control.

4.2.4 HISTOLOGY RESULT OF THE NORMAL PANCREAS

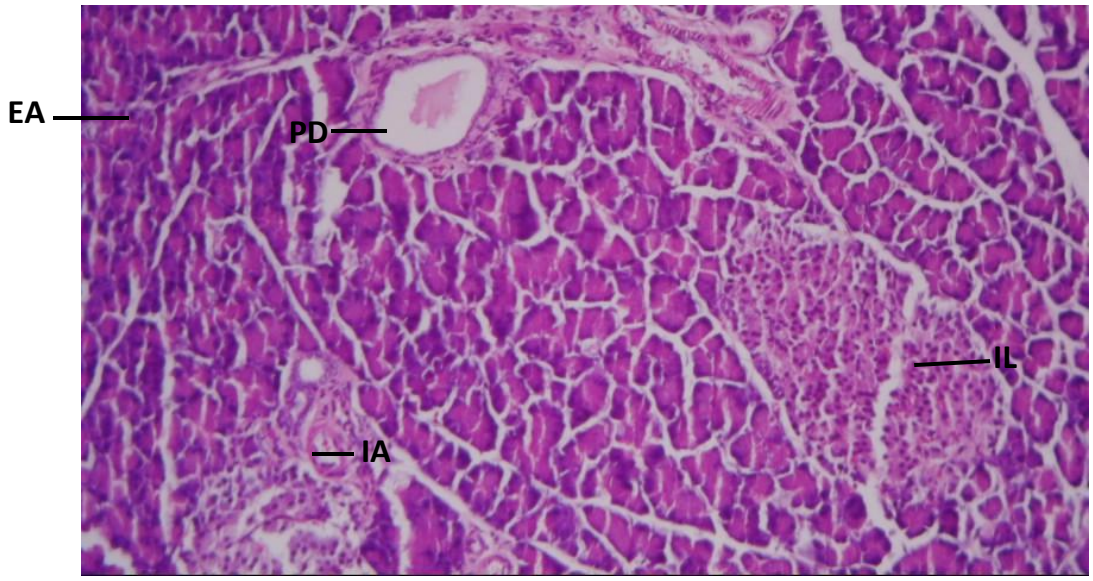

Fig 4.1: Rat Pancreas Control. Showing normal architecture composed of: Exocrine acini (EA), pancreatic duct (PD), interlobar arteries (IA), islets of Langerhans (IL): H&E x 100

4.2.5 THE PANCREAS AFTER ADMINISTRATION OF 150mg/kg DOSE OF EXTRACT

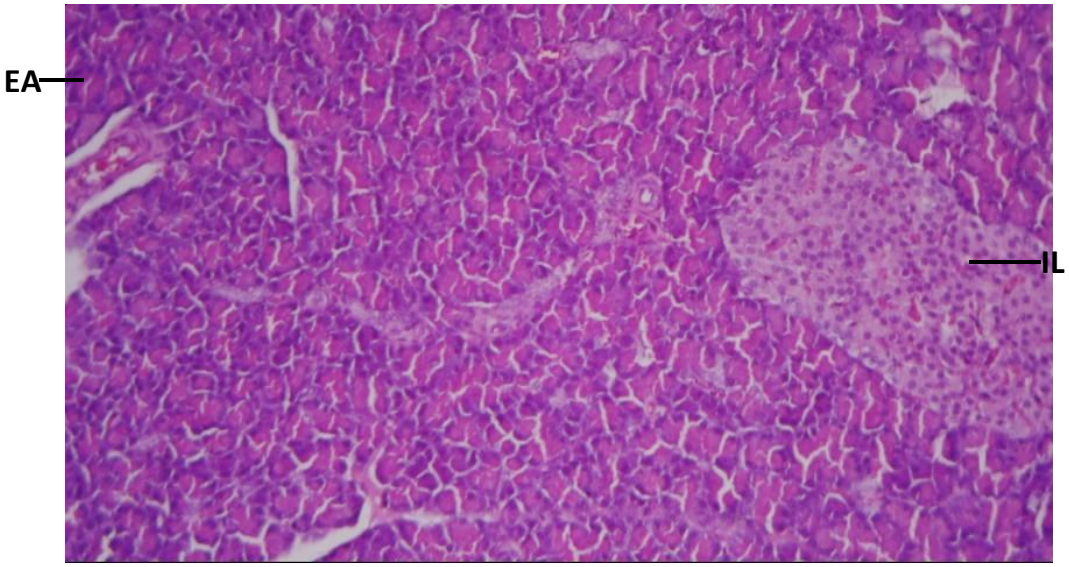

Fig 4.2: Rat pancreas given 150mg aqueous Extract showing normal acini

(EA), and active and proliferating islets (IL): H&E x 100

4.2.6 THE PANCREAS AFTER ADMINISTRATION OF 300mg/kg DOSE OF EXTRACT

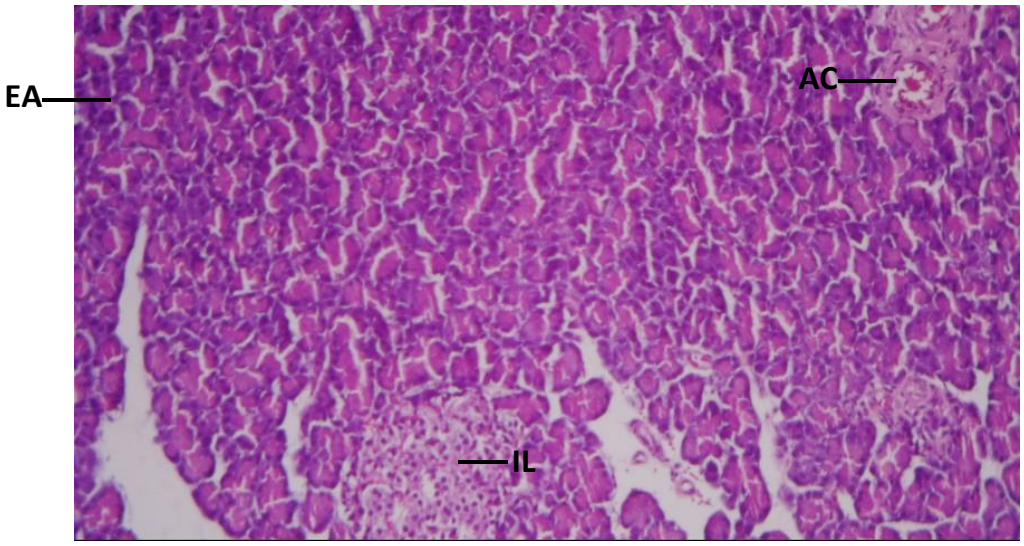

Fig 4.3: Rat pancreas given 300mg aqueous Extract showing: normal exocrine acini (EA) and islets of Langerhans (IL) and mild active stromal congestion (AC): H&E 100

4.2.7 THE PANCREAS AFTER ADMINISTRATION OF 800mg/kg DOSE OF EXTRACT

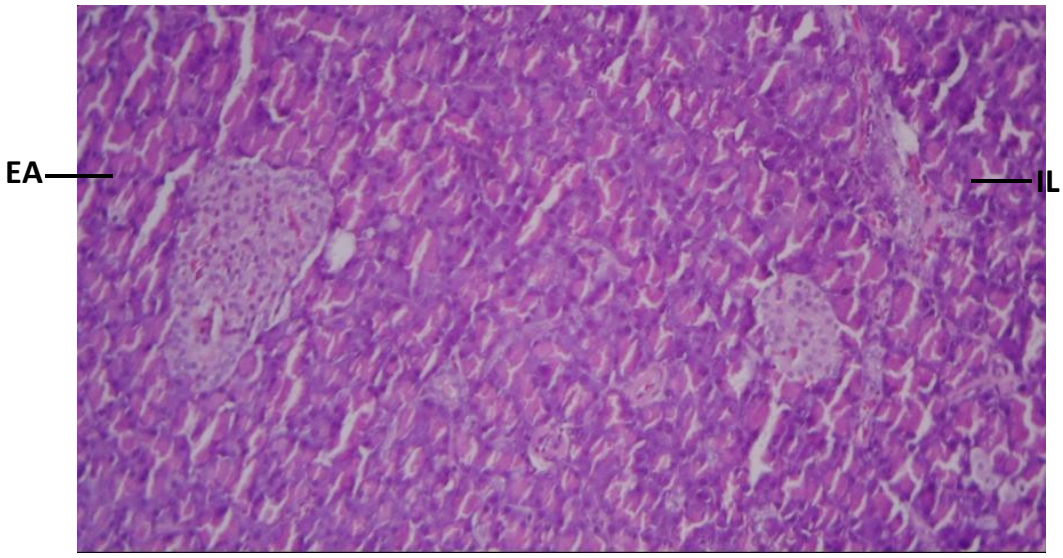

Fig 4.4: Rat pancreas given 800mg aqueous Extract showing: normal acini (EA)

and islets of Langerhans (IL): H&E x 100

4.2.8 THE PANCREAS AFTER ADMINISTRATION OF 2000mg/kg DOSE OF EXTRACT

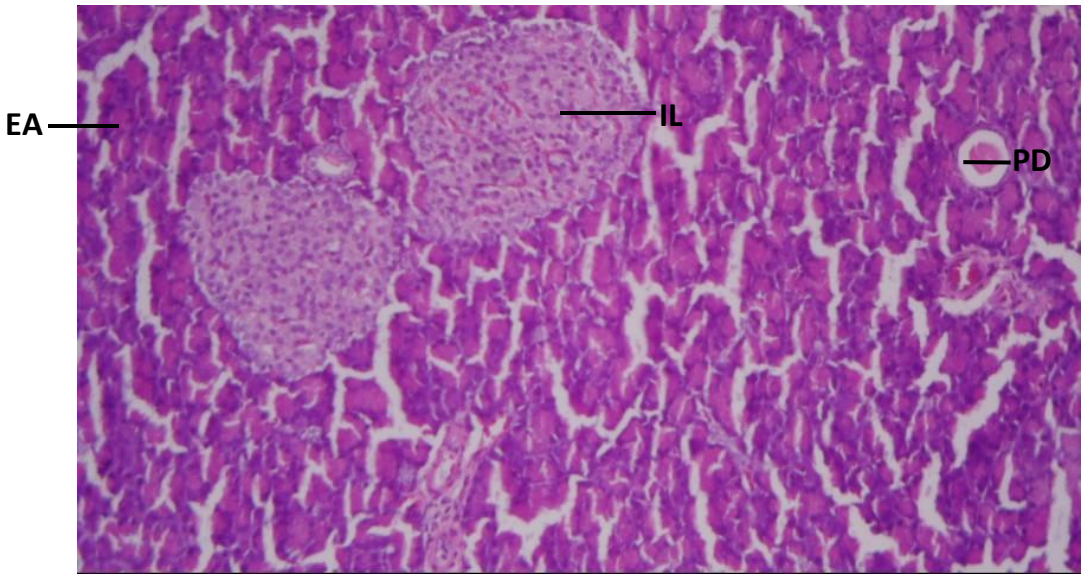

Fig 4.5: Rat pancreas given 2000mg aqueous Extract showing: normal exocrine acini (EA), proliferating islets of Langerhans (IL) and dilated pancreatic duct (PD): H&E x 100

4.2.9 THE PANCREAS AFTER ADMINISTRATION OF 5000mg/kg DOSE OF EXTRACT

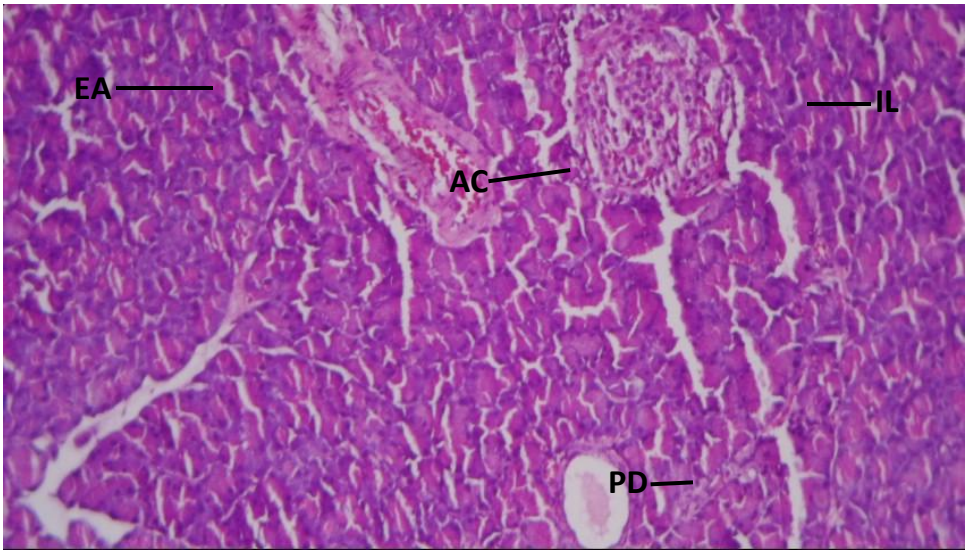

Fig 4.6: Rat pancreas given 5000mg aqueous Extract showing: normal exocrine acini (EA) and islets of Langerhans (IL), active stromal congestion (AC) and dilated pancreatic duct (PD): H&E x 100

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1 DISCUSSION

Several research has explored the potential benefits of various parts of *Picralima nitida*, this study particularly focuses on the effect of its aqueous stem bark extract on insulin and glucose levels in normal Sprague-Dawley rats. Ethnomedicine involves the use of medicinal plant for treatment of diseases. It is important to know that a beneficial substance can have a harmful effect as well, when not consumed in the right proportion. Therefore, in the course of this study both acute and subchronic toxicity tests were carried out. Acute toxicity study was carried out in 2 phases and this lasted for two (2) weeks. For the phase I acute toxicity test the rats were given 10mg, 100mg and 1000mg dose of extract per kg body weight while rats in phase II received 1600mg, 2900mg, 5000mg of extracts. There were no visible signs of toxicity such as shivering, rash, lacrimation, drooling, difficulty in breathing, sluggish movement or death observed in all the animals administered the extracts. Although according to the review on Medicinal uses, phytochemistry and pharmacology of *Picralima nitida* in tropical diseases by Erharuyi *et* al., (2014), the methanol fruit rind extract of *P. nitida* administered to the rats had some toxic effects on the liver, kidneys and the lungs after prolonged exposure at doses (1.5- 6g/kg) with LD_{50} values of 14.5 and 12.5 g/kg for male and female rats respectively which was marked by elevated serum aspartate activity. This suggests that the stem bark extract of *P. nitida* may be more likely safe for short term use at the tested doses and probably more effective to the body in the long run.

In the subchronic toxicity tests, the aqueous stem bark extract of *P. nitida* reduced blood glucose levels when the baseline fasting blood sugar (FBS) was compared with the 4th week FBS result. In the rats administered 150mg/kg extract, there was an initial increase after one week, then a slight fluctuation and finally a decrease in FBS by the fourth week, compared to normal control. Those given 300mg/kg extract, generally showed a decrease in FBS levels throughout the study when compared to normal control. In those administered 800mg/kg extract, fasting blood glucose decreased till the final week, compared to the normal control. In those given 2000mg/kg extract, there was a slight increase followed by a peak in the second week, and then a general decrease in FBS compared to normal control. Those administered 5000mg/kg extract showed a decrease in FBS compared to normal control. Statistically, the p-values indicate that there are no significant differences in the FBS levels across the groups at any time point, as all p-values are > 0.05.

The aqueous stem bark extract of *Picralima nitida* stimulated insulin secretion substantially only at the 5000mg/kg dose. However, this increase is not statistically significant based on the provided p-value of 0.433, as this p-value suggests that despite the observable increase at the 5000mg/kg dose, it is not statistically confirmed as a significant change compared to the control and other doses. Insulin secretion at other doses (150mg/kg, 300mg/kg, 800mg/kg, and 2000mg/kg) showed no significant change compared to the normal control.

There was a statistically significant decrease in the weight of the pancreas from the normal control (0.54 ± 0.02) to all other doses (0.34 ± 0.02 to 0.36 ± 0.02). The decrease is consistent across the 150mg/kg to 2000mg/kg doses, with a slight but statistically significant increase at the

highest dose of 5000mg/kg which is still significantly lower than the control. The reduction is most pronounced at the 300mg/kg dose.

The aqueous stem bark extract of *P. nitida* at 2000mg/kg dose increased Islets of Langerhans markedly. At 150mg/kg and 800mg/kg dose, there was also an increase in the Islets while at 300mg/kg and 5000mg/kg there was a decrease in Islets of Langerhans. In all the administered doses there was no sign of inflammation on the pancreas.

5.2 CONCLUSION

The aqueous stem bark extract of *Picralima nitida* demonstrates potential as a hypoglycemic agent, without significantly altering insulin secretion. While it significantly reduces pancreatic weights, no visible sign of toxicity was observed in the rats in acute toxicity tests during our experimental period and there was no harmful effect of the extract on the pancreas during this period, suggesting that the doses administered maybe safe for use. These findings also suggest that *Picralima nitida* aqueous stem bark extract could be a promising candidate for managing blood sugar levels, therefore, further research is necessary to explore its long-term safety and efficacy, as well as the mechanism behind its effects in the blood sugar and pancreatic weight.

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APPENDIX I

WEIGHT OF THE RATS AND AMOUNT OF EXTRACT ADMINISTERED DURING ACUTE TOXICITY TEST- PHASE 1

GROUPS	DOSAGE (mg/kg)	WEIGHT (g) OF EACH RAT AND AMOUNT OF EXTRACT ADMINISTERED (ml)
I (Control)	Pellet feed and water only	
II	10mg/kg body weight	Rat a (198g)- 1ml Rat b (193g)- 0.975ml Rat c (190g)-0.960ml
III	100mg/kg body weight	Rat a (178g) - 1ml Rat b (173g) - 0.972 Rat c (172g) -0.966
1V	1000mg/kg body weight	Rat a (154g) - 0.981 Rat b (157g) - 1ml Rat c (155g) - 0.987

WEIGHT OF THE RATS AND AMOUNT OF EXTRACT ADMINISTERED DURING ACUTE TOXICITY TEST- PHASE 2

GROUPS	DOSAGE (mg/kg)	WEIGHT OF EACH RAT AND AMOUNT OF EXTRACT ADMINISTERED (ml)
I(Same control in phase 1)	Pellet feed and water only	
II	1600mg/kg body weight	Rat a (153g) - 0.2448ml
III	2900mg/kg body weight	Rat b (152g) - 0.4408ml
1V	5000mg/kg body weight	Rat c (239g) - 1.195ml

CALCULATION FOR EXTRACT REQUIRED FOR ADMINISTRATION

To calculate dosage: Rat 1 = Standard dose/ 1000 × weight of rat

Therefore, dry extract can be calculated using the formula: Standard dose/1000 × weight of rat in gram.

EXAMPLE:

Group II-10mg/kg body wt (i.e standard dose=10mg/kg)

Rat a (198g) = 10/1000 × 198 = 1.98mg of dry extract.

Rat b (193g) = 10/1000 × 193 = 1.93mg of dry extract.

Rat c (190) = 10/1000 × 190 = 1.90mg of dry extract.

Total amount of dry extracts = 1.98mg + 1.93mg + 1.90mg = 5.81mg of dry extract.

Thus, Rats a, b and c in group II would be required to take a total of 5.81mg of the dry extract.

CALCULATION FOR WATER REQUIRED TO DISSOLVE THE EXTRACT

The highest amount of extract was diluted with 1ml of water. The rat that is to receive the highest amount of extract is then used as a standard to determine the dose to be administered to other rats in the group.

Quantity Of Extract ÷ Highest Extract Value

EXAMPLE:

For group II:

NOTE: If 1.98mg of rat require 1ml of distilled water

Then xmg will require x/1.98

Rat a: requires 1ml of distilled water (that is 1.98 / 1.98).

Rat b: requires – 1.93/1.98 = 0.975ml of distilled water

Rat c: requires – 1.90/1.98 = 0.960ml of distilled water

CALCULATION FOR STOCK IN ACUTE TOXICITY TEST

Total amount of distilled water to be used =1 + 0.975 + 0.960 = 2.935ml.

Thus, 2.935ml of distilled water was used in the dissolution of 0.00581g of total extract.

The ml of diluted extract that was administered to the test animals were:

Rat a - - - - 1ml

Rat b - - - - 0.975ml

Rat c - - - - 0.960

NOTE: For acute toxicity test the animals were dosed once.

APPENDIX II

CALCULATION FOR STOCK IN SUBCHRONIC TOXICITY TEST

Sum of the extract values in same group × 7 days

Sum of the values obtained for water in the same group × 7 days

EXAMPLE

For group II in subcronic toxicity test:

112.35mg × 7 = 786.45mg = 0.78645g (Stock for 7 days)

Total amount of distilled water to be used =0.987+ 1+ 0.974+0.981+0.916 = 4.865ml daily. For 7 day - 7×4.865 = 34.055ml ≈ 34ml

Thus, 34ml of distilled water was used in the dissolution of 0.78645g of total extract and 0.987ml, 1ml, 0.981ml, 0.981ml and 0.916ml of the extract was administered daily to head, leg, hand, tail and plain rats respectively for seven (7) days.

Weight (g) of the test rats for week one (1)

	Group I	Group II	Group III	Group IV	Group V	Group VI
Head rat	224	152	175	190	190	218
Leg rat	217	154	180	190	197	220
Hand rat	213	151	178	194	182	230
Tail rat	214	151	165	198	159	214
Plain rat	148	141	165	199	159	254

AMOUNT OF EXTRACTS (in ml) ADMINISTERED TO TEST ANIMALS DAILY IN WEEK 1

	Group II	Group II	Group IV	Group V	Group VI

Head rat	0.987	0.972	0.955	0.964	0.858
Leg rat	1	1	0.955	1	0.866
Hand rat	0.981	0.988	0.975	0.924	0.906
Tail rat	0.981	0.917	0.995	0.807	0.843
Plain rat	0.916	0.917	1	0.807	1

AMOUNT OF EXTRACTS (in ml) ADMINISTERED TO TEST ANIMALS DAILY IN WEEK 2 AND THEIR WEIGHT (in g)

	Group II	Ml	Group III	Ml	Group IV	ml	Group V	ml	Group VI	ml
Head rat	200	0.917	217	0.923	195	0.813	197	0.943	220	0.789
Leg rat	218	1	195	0.830	212	0.883	209	1	245	0.878
Hand rat	195	0.894	235	1	240	1	201	0.962	259	0.928
Tail rat	170	0.780	200	0.851	221	0.921	186	0.890	262	0.939
Plain rat	182	0.835	192	0.817	222	0.925	162	0.780	279	1

AMOUNT OF EXTRACTS (in ml) ADMINISTERED TO TEST ANIMALS IN WEEK 3 AND THEIR WEIGHT (in g)

	Group II	Ml	Group III	Ml	Group IV	ml	Group V	ml	Group VI	ml
Head rat	246	1	234	0.897	205	0.813	222	0.895	223	0.820

Leg rat	214	0.870	205	0.785	225	0.893	248	1	245	0.901
Hand rat	225	0.915	261	1	252	1	239	0.964	258	0.949
Tail rat	179	0.727	219	0.839	222	0.881	219	0.883	272	1
Plain rat	193	0.785	178	0.682	221	0.877	147	0.593	270	0.993

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AMOUNT OF EXTRACTS (in ml) ADMINISTERED TO TEST ANIMALS IN WEEK 4 AND THEIR WEIGHT (in g)

	Group II	Ml	Group III	Ml	Group IV	ml	Group V	ml	Group VI	ml
Head rat	274	1	244	0.887	230	0.836	246	0.895	244	0.787
Leg rat	234	0.854	207	0.753	243	0.884	275	1	259	0.835
Hand rat	260	0.949	275	1	275	1	266	0.967	262	0.845
Tail rat	193	0.704	240	0.873	242	0.880	252	0.916	310	1
Plain rat	201	0.734	207	0.753	246	0.895	139	0.505	269	0.868

APPENDIX III

INSULIN AND GLUCOSE READINGS

Baseline fasting blood glucose (FBG) of the test animals in mg/dl

	Group I	**Group II**	**Group III**	**Group IV**	**Group V**	**Group VI**
Head rat	97	82	71	81	102	105
Leg rat	96	93	100	90	100	81
Hand rat	98	78	69	98	99	78
Tail rat	88	81	74	99	86	109
Plain rat	72	83	68	83	63	107

Fasting blood glucose (FBG) in mg/dl of the test animals for week one (1)

	Group I	Group II	Group III	Group IV	Group V	Group VI
Head rat	86	66	58	69	109	70
Leg rat	69	88	61	82	98	62
Hand rat	97	92	78	70	78	66
Tail rat		116	82	85	93	81
Plain rat	58	126	70	75	76	87

Fasting blood glucose (FBG) in mg/dl of the test animals for week two (2)

	Group I	Group II	Group III	Group IV	Group V	Group VI
Head rat	81	84	68	107	88	111
Leg rat	64	91	60	70	101	85
Hand rat	126	73	63	56	88	65
Tail rat	62	68	77	75	75	64
Plain rat	61	90	73	83	153	78

Fasting blood glucose (FBG) in mg/dl of the test animals for week three (3)

	Group I	Group II	Group III	Group IV	Group V	Group VI
Head rat	79	94	59	62	59	64
Leg rat	81	109	60	92	87	53
Hand rat	84	76	77	62	65	63
Tail rat	75	73	86	43	80	61
Plain rat	80	78	78	69	54	77

Fasting blood glucose (FBG) in mg/ dl of the test animals for week four (4)

	Group I	Group II	Group III	Group IV	Group V	Group VI
Head rat	64	73	61	71	69	71
Leg rat	81	72	65	79	71	72
Hand rat	69	81	72	80	69	75
Tail rat	83	60	103	71	69	72

Plain rat	70	70	63	68	61	79

Values for insulin levels obtained

	Group I	Group II	Group III	Group IV	Group V	Group VI
Head rat	0.513	2.500	2.760	5.000	2.760	2.500
Leg rat	1.025	11.041	2.264	4.529	8.72	6.730
Hand rat	0.625	2.264	5.520	3.715	1.682	5.000
Tail rat	14.010	1.380	5.520	3.048	1.857	97.521
Plain rat						