LIPID PROFILE AND TOXICOLOGICAL EVALUATION OF AQUEOUS AND METHANOL EXTRACTS OF *Acalypha Wilkesiana* LEAF IN WISTAR RATS.

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UNIVERSITY OF BENIN, BENIN CITY.

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A THESIS WRITTEN IN THE DEPARTMENT OF MEDICAL BIOCHEMISTRY AND SUBMITTED TO THE SCHOOL OF POST GRADUATE STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF SCIENCE (M.SC.) MEDICAL BIOCHEMISTRY OF THE UNIVERSITY OF BENIN, BENIN CITY.

OCTOBER, 2023.

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External Examiner **Date**

Dedication

I dedicate this project work to God Almighty for His wisdom, guidance and protection over my life all through my stay in school, my lovely parents Mr. and Mrs. Dickson Iyamu for their undying love and support and my siblings for their love and prayers.

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ABSTRACT

The experiment is aimed at carrying out a toxicological study on rats given different doses of both aqueous and methanol extract of *Acalypha Wilkesiana* and to determine the degree of toxicity on some organs of the rats fed with both aqueous and methanol extract of *Acalypha Wilkesiana*. A total of seventy five male albino rats were used for the whole of the experiments. For Acute toxicity study phase one of the studies alone was carried out.

For sub-acute toxicity study: The rats were placed into 4 different groups and group 1 served as the control group that was fed with pellets and water, the other groups that's group 2 to 4 was divided into 2 sub-groups (group 2A aqueous and 2B methanol), and was given different doses of plant extracts. For 28 days, to mark the end of our sub-acute toxicological study, liver and kidney function biomarkers, electrolytes, lipid profile assays were investigated. For the acute toxicity study, it was observed that there was no visible sign of toxicity or death observed in all the animals administered with the aqueous and methanol extract of *Acalypha Wilkesiana*.

From the sub-Acute toxicity study, it was observed that the activity of aspartate aminotransferase (AST) was increased following the administration of graded doses of the methanol extract when compared with normal control but for aqueous extract the activity was not significantly increased with a significant difference ($p > 0.05$).The activity of alanine aminotransferase (ALP) was not significantly impacted by the graded dose of the methanol and aqueous extract relative to the normal control ($p > 0.05$). However, the concentration of direct bilirubin and albumin was significantly ($p < 0.05$) elevated in a dose-dependent manner in groups exposed to both methanol and aqueous extract relative to the normal control when compared to the control. The sub-acute effect of the methanol extract of *Acalypha wilkesiana* on the Electrolyte levels in male rats, at the

dosage of 1000 mg extract / Kg body weight of rat, the concentration of plasma sodium ion significantly reduced relative to the normal control ($p < 0.05$), at the highest dose of 2000 mg extract/ Kg body weight of rat, there was a significant decrease in the concentration of potassium relative to the normal control ($p < 0.05$). Graded dose administration of the aqueous extract of *Acalypha wilkesiana* did not significantly increase or decrease the concentration of plasma electrolyte levels in the rat when compared to the normal control ($p > 0.05$). An increase in the dose of the methanol extract did not significantly increase or decrease ($p > 0.05$) the concentration of urea and creatinine in all the treated groups when compared to the control. An increase in the dose of the aqueous extract to 2000 mg/Kg bodyweight led to a significant decrease ($p > 0.05$) in the concentration of urea and creatinine when compared to the control in a dose-dependent manner. The administration of both aqueous and methanol extract significantly elevated the concentrations of T. CHOL, and LDL-cholesterol but inversely impacted the concentration of HDL-cholesterol in a dose-dependent manner relative to the control ($p < 0.05$). However, the concentration of TG and VLDL-cholesterol increased, but not with a significant difference ($p > 0.05$).

From the study carried out, the results revealed that it is more likely that the administration of aqueous and methanol leaves extracts of *Acallypha wilkiasiana* do not have any observed acute toxicity effect. However, the sub- acute toxicity study showed mild effect on increase of doses of the extracts which could be as a result of prolonged administration.

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CHAPTER ONE

1.0 INTRODUCTION

In both industrialized and developing countries, ethno medicinal plants have been employed for ages as therapeutic treatments for the treatment, mitigation, and prevention of ailments. The common perception is that medicinal plants are natural products devoid of synthetic preservatives and so it's safe for discretional uses. Over 85% of disease conditions of humans and animals, ranging from bacterial infections to cancer and immunological disorders are treated with either natural products or compounds gotten from natural products (Newman and Cragg, 2016). Nearly 70% of therapeutic compounds in use today or presently in clinical trials were obtained directly from natural products or synthesized from natural products (Newman and Cragg, 2016). As a result, the natural environment has been recognized as a valuable source of distinctive biodiversity for the identification of pharmaceutical lead compounds and drug discovery (Harvey *et al.,* 2015). Drug discovery process is complicated and interwoven, requiring not only information about pharmacodynamics and pharmacokinetic parameters of the compound but also, more importantly, its safety (Thomford *et al.,* 2018). Drug-induced liver injury and nephrotoxicity which are the major causes of pharmaceutical withdrawals of promising drug candidates in clinical trials (Schnellman 2008 and Weiler *et al.,* 2015).

Acalypha wilkesiana, with common names copperleaf and Jacob's coat (RHS Plant Finder 2017) is an evergreen shrub growing at 3 meters (9.8 feet) high and 2 meters (6 feet 7 inches) across. It has a closely arranged crown, with an erect stem and numerous branches. Both the branches and the leaves are covered in fine hairs. The leaves are wide and broad with teeth around the edge, even though they may be flat or crinkled. The leaves can range in size from 3.9 to 7.9 inches (or

10 to 20 centimeters) long and 15 centimeters (5.9 inches) broad. The leaves have a mottled appearance due to their coppery green color and crimson splotches. *Acalypha wilkesiana* is a tropical and subtropical plant that grows naturally in Vanuatu and occurs in the Pacific Islands. It prefers light well drained soil and is suited to a protected shady position. It can be damaged by both drought and frozen dew. It needs a minimum temperature of above 10 °C (50 °F). It is best suited to hardiness zones 10-12.

The chemical compounds known as secondary metabolites which are active principles of many drugs and have been reported to possess antimicrobial, anti-inflammatory, anticancer, anti-viral, anti-malarial and antifungal properties (Olubodun *et al.,* 2007).

Young plant shoots without blossoms are consumed as cooked vegetables. To treat diarrhea and dysentery, *Acalypha wilkesiana* leaves are squeezed into water. The resulting juice is then consumed (WHO 2009). The juice of fresh leaves of *Acalypha wilkesiana* is drunk as a treatment for laryngitis. They are chewed on as a first-aid treatment for a ruptured appendix (WHO 2009).The fresh shoots are squeezed into water and the solution gotten is drunk to regulate menstruation and as an abortifacient (Presumably this last treatment is a much stronger juice than that used for diarrhea (Ken Fern 1997). The leaves can also be applied externally, as young shoots are used to treat skin rashes (Tolu *et al.,* 2007). The leaves are boiled in water and then used to massage patients with fevers (WHO 2009).

Nature has provided medicinal plants with metabolites to protect them from potential attacks from animals and the environment, even though they are often thought of as safe medications with a broad margin of safety. Some of these plants' coloring, scent, and toxicity are caused by these metabolites (Weng *et al.,* 2012). It is, therefore, important to study their safety margins.

Therefore my goal is to investigate *Acalypha wilkesiana* aqueous and methanol plant extract for safety in acute and sub-acute toxicity study used in male wistar rats.

1.1 Justification of study

Despite the fact that medicinal plants are frequently utilized and have a promising therapeutic potential, ingestion of these plants and the products they produce is rarely tracked and is therefore still unconfirmed for symptoms of toxicity. The knowledge of the potential effects of plants with medicinal value is limited hence difficulty in identifying the safety and effectiveness (WHO, 2002). Exhaustive researchers have demonstrated that long term administration of plant extracts in animal models may lead to bioaccumulation that could result to toxicity (Van Andel *et al.,* 2012). Thus the, toxicology reports and careful use of medicinal plants is frequently encouraged to avoid undesirable toxicity.

The leaves of *Acalypha wilkesiana* plant are eaten as vegetables for the management of hypertension (Ikewuchi *et al.,* 2008). The juice gotten from the leave is used for the treatment of gastrointestinal disorder and fungal infections. Boiling leaves of *Acalypha wilkesiana* are applied to the bodies of feverish individuals in the Central Province. But the dosage of the leaf extract is unknown. Aphids, mites and scales are pest and disease problems associated with *Acalypha wilkesana* plant (Edward, 2014).

(Oladunmoye 2006) reported that the presence of saponins, tannins, anthraquinone and glycoside in the leaves of *Acalypha wilkesiana*, has antifugal and antibacterial properties (Oladunmoye, 2006, Ogundiani, 2005). (Hanna *et al.,* 2013) established that prolonged oral use of *Acalypha wilkesana* at high dose may be toxic. The current study is thus to examine the Acute and sub-acute toxicity of *Acalypha wilkesana* in Wistar healthy male rats.

1.2 **Aim and objectives**

The study was aimed at determining the level of toxicity on the heart, kidney and liver of rats fed with both aqueous and methanol leaf extract of *Acalypha Wilkesiana* using some specific Biochemical assays.

1.2.1 **Specific Objectives of study**

Evaluation of the Acute and sub-acute toxicity of methanol and aqueous extracts of *Acalypha wilkesana* leaf in healthy Wistar rats and to assess the effect of the long-term administration of *Acalypha wilkesana* extracts on some biochemical parameters (Electrolites, LFT, Lipid profile, Renal function).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 MEDICINAL PLANTS

Medicinal plants, also refer to as medicinal herbs, have been discovered and used in traditional medicine practices since prehistoric times. Numerous chemical compounds are produced by medicinal plants for a variety of purposes, such as defense against pathogens, illnesses, insects, fungus, and herbivorous mammals. Numerous phytochemicals have been shown to have both potential and proven biological activity. The results of using a complete plant as medication are unclear, though, because a single plant includes a vast variety of phytochemicals. Furthermore, despite extensive scientific investigation to establish efficacy and safety, the phytochemical content and pharmacological effects of many plants with therapeutic promise remained unreachable (Ahn, 2017).

Therefore those plants that possesses the ability to exert pharmacological effects and have therapeutic properties that are beneficial to the human body are generally called Medicinal Plants (Rios *et al.,* 2005), these plants synthesize and accumulate secondary metabolites naturally such as Terpenes, Flavonoids, Tannins, Resins, Saponins, Quinines, Alkaloids, Sterols etc (Rasoon, 2012a).

The term "medicinal plants" refers to a variety of plant species used in herbalism, some of which have medicinal properties. As the "backbone" of traditional medicine, these plants are regularly used by more than 3.3 billion people in less developed nations (Davidson-Hunt, 2000). Medicinal plants are considered to be a rich resource of ingredients which can be used in drug development and synthesis. The use of traditional medicine and medicinal plants in most

developing countries, as a bases for the maintenance of good health, have been widely observed by UNESCO, 1996. During the past decade, traditional systems of medicine have become a topic of global importance. According to recent estimates, a significant section of the population in many developing nations relies significantly on traditional healers and medicinal herbs to address their basic medical needs. Although there may be access to modern medicine in some nations, herbal medicines, or phytomedicines, have frequently maintained their appeal due to historical and cultural factors. Active compounds that are employed in the manufacture of various medications are commonly extracted from medicinal plants using them as raw materials. Like in case of laxatives, blood, thinners, antibiotics and anti-malarial medications, contain ingredients from plants. Moreover the active ingredients of Taxol, vincristine, and morphine isolated from foxglove, periwinkle, yew, and opium poppy, respective. Medicine, in several developing countries, uses local traditions and beliefs, are still the mainstay of health care. As defined by WHO, health is a state of complete physical, mental, and social wellbeing and not merely the absence of disease or infirmity (Lucy and Edgar, 2000).

According to Nwachukwu *et al.,* (2010); The basic products of photosynthesis, which are carbohydrates, proteins, and triglycerides (fats and oils), are believed to play a crucial role in the role of food crops, which account for the majority of human nutrition. The majority of medications, herbs, traditional remedies, essential oils, and cosmetics are made from secondary plant metabolic products such alkaloids, terpenoids, and flavonoids. These compounds, which are regarded as a huge chemical library of biological systems, have evolved as reactions of plants to stress, predation, and competition. Therefore, it is normally “extracts” not the plants themselves or their parts such as fruits, seeds leaves etc, that are used for medicinal effects. However, medicinal plants possess the so-called pathological niche attribute and take on

pathogenic structure. According to how a medicinal herb affects human physiology, it can therefore be utilized to treat a variety of diseases.

2.2 **Importance of medicinal plants**

Pharmacological treatment of disease started long time ago with the use of herbs (Sandberg *et al*., 2001). Basically, herbal remedies are a widespread feature of folk healing practices all around the world. Some of these customs are succinctly documented, giving instances of an imposing collection of significant world-wide herbal therapeutic practices. It also stems from the widely-held notion that some herbal medicines' positive effects are caused by the synergistic fusion of a number of active components (Taiz and Zeiger, 1991).

Introduction of plant derived drugs in modern medicine has been associated with the use of plant derived materials as an indigenous cure in traditional system of medicine (Igoli *et al*., 2003). Some of these plants have been found to possess significant antibacterial, antifungal, anticancer, antidiuretic, anti-inflammatory and anti-diabetic properties (Adelowo and Oladeji, 2016).

Common uses of these herbal medicines are in venom neutralization by lupeol acetate isolated from the root extract of *Hemidesmus indicus* (Chatterjee, 2006), treatment of hypertension and lowering of blood sugar by serpentine isolated from the root of *Rauwolfia serpentine*, treatment of Hodgkin's, choriocarcinoma, non-Hodgkin lymphomas, leukemia in children, testicular and neck cancer from vinblastine isolated from the *Catharanthus rosesus* (Farnsworth and Bunyapraphatsara, 1992), treatment lymphocytic leukemia in childhood advanced stages of Hodgkin's, lymophosarcoma, cervical and breast cancer amongst others. Drugs derived from plants are used to cure mental illness, skin diseases, tuberculosis, diabetes, jaundice, hypertension and cancer.

2.3 Safety and toxicity of medicinal plants

2.3.1 Safety of Medicinal plant

Many fans of herbal medicines argue that products with a long history of popular use are generally safe when used properly at common therapeutic doses (Fong, 2002). A very important question underlying this statement is, to what extent to which the absence of evidence of toxicity could be taken as evidence of the absence of toxicity or safety of herbal medicines. It relies on the sort of toxic impact and the likelihood of finding such a negative consequence under the conditions present in the traditional usage to determine whether the absence of records of adverse effects is a sign of lack of toxicity. Herbal medicine is likely to be detected and linked to acute symptoms and short-term harmful consequences, such as gastrointestinal problems and dermatological abnormalities. Therefore, the absence of such findings offers some proof that these specific endpoints are safe. However, unless a properly designed epidemiology study (preferably a prospective cohort study) is carried out, it is unlikely that long-term adverse outcomes, such as cancer, liver and kidney damage, reproductive dysfunctions, birth defects, and several morbidities that are more difficult to detect, will be associated with the widespread use of a medicine. Therefore, the lack of proof of these negative effects in the context of traditional consumption of herbal medicines does not rule out the possibility that they could still occur. As far as drugs are concerned, safety is assumed only when the null hypothesis (absence of toxicity) has not been disproved after being challenged by properly designed and comprehensive set of pre-clinical and clinical studies that had enough statistical power to reject it if it were false.

2.3.2 Toxicity and Medicinal plants

The toxicity of the plant extracts was reviewed at cellular and whole organism level. According to Horvath (Horvath, 1980), Cytotoxic substances hinder cellular adhesion, result in pronounced morphological alterations, impair replication rates, or lower overall viability. However, the extent to which the chemicals produce cytotoxicity, how long the cells are exposed to them, and the kind of medicine being evaluated all influence how dangerous they are to cells (Riss and Moravec, 2004; Di Nunzio *et al.,* 2017). Although medicinal plants are generally considered to be safe, they are not entirely free of side effects or toxicity (Boukandou *et al.,* 2015).The chemical makeup of a medicinal plant affects its toxicity in several ways. Even when using extracts with low toxicity, traditional herbal remedies may still be hazardous after acute or chronic exposure. The plant extracts could also be mutagenic or carcinogenic (Ferreira-Machado *et al.,* 2004). Despite the advantages of medicinal plants, some could pose a risk to the users' health due to possible negative side effects or adverse reactions that could be brought on by toxic or excessive amounts. Acute toxicity and patient mortality could result from this (Tamokou and Kuete, 2014; Schultz *et al.,* 2020).

Human liver and kidney disorders are linked to the usage of traditional herbal remedies. The toxicity of some therapeutic herbs to various organs has also been demonstrated in a number of animal experiments. Various organ systems can be affected by toxic plants, and some plants have numerous toxic principles that can influence multiple organ systems. (Tamokou and Kuete, 2014).

2.3.3 Acute toxicity

The term "acute toxicity" refers to a substance's negative effects that might happen after either a single exposure or several exposures over a short period of time (often less than 24 hours).If adverse reactions take place within 14 days following a substance's ingestion, they are considered to be acute toxic consequences. The detrimental health effects from repeated exposures to a chemical over a longer time (months or years), frequently at lower levels, are referred to as chronic toxicity, as opposed to acute toxicity.

It is generally agreed upon that using humans as test subjects for studies on acute (or chronic) toxicity is unethical. Investigating unintentional human exposures (such as accidents in factories) can provide some information, though. Otherwise, the majority of evidence on acute toxicity comes from experiments on animals or, more recently, from in vitro testing techniques and extrapolation from information on compounds that are related (Walum, 1998).

2.3.4 Sub-acute toxicity

Sub-acute toxicity tests are performed to assess a new drug's potential side effects after a 2-4 week therapy term. To choose the dosage levels to be employed in later sub-chronic and chronic toxicity tests, range-finding sub-acute toxicity experiments are carried out. Additionally, preliminary clinical trials with treatment periods of up to 4 weeks may be supported by sub-acute toxicity investigations. These investigations are intended to evaluate the development and remission of drug-induced lesions, but they typically last too little time to fully characterize all potential side effects that can manifest during prolonged clinical usage or during chronic toxicity and carcinogenicity testing (Colerangle 2017) .

2.5 *Acalypha wilksiana*

Figure 1.1: *Acalypha wilkesiana* plant

2.5.1 Description

Acalypha wilkesiana is a plant commonly called Irish petticoat, it is native to the south pacific islands and belongs to the family Euphorbiaceae. The plant has antimicrobial and antifungal properties and in traditional medicine, the leaves are eaten as vegetables in the management of hypertension, being a diuretic plant. *Acalypha wilkesiana* is a plant of great ornamental value due to its showily colored foliage and is widely cultivated in the tropical and subtropical countries (Omage and Azeke, 2014). *Acalypha wilkesiana* is extensively used in traditional medicine to manage or cure hypertension, either alone or as a key ingredient in many herbal medicines.

The health of individuals and communities depends greatly on this therapeutic plant. Some chemical constituents in this plant have medical benefits because they have defined physiological effects on people (Ekhaise *et al.,* 2010, Jeruto *et al.,* 2011). The most important of these

bioactive constituents of the plant are alkaloids, tannins, flavonoids and phenolic compounds (Dabai *et al.,* 2013, Muhammad *et al.,* 2013).

Acalypha wikesiana is one of those ethno medicinal plants with health benefits. *Acalypha wikesiana* is a plant (shrub) found worldwide mostly around the tropical of Africa, America and Asia. Its common names are copperleaf and Jecob's coat and it is one of the most widely known and utilized of the family Euphorbiacaece. *Acalypha wilkesiana* is an evergreen shrub usually planted around homes for horticultural purposes. The plant may grow up to 3meters high with erect stems and many branches. Previous scientific evaluation of *Acalypha wilkesiana* leaves revealed mycotic/antifungal activity (Oyelami *et al.,* 2003) and some level of liver toxicity conducted after treatment for 28 days (Olukunle *et al.,* 2014). It looks its best when provided with regular watering during drought and will grow on a wide variety of garden soils, easily or boiled decoction is used for the treatment of gastrointestinal disorder and fungal infections. Aphids, mites and scales are pest and disease problems on *Acalypha wilkesana* plant (Edward, 2014); Oladunmoye (2006) reported the presence of saponins, tannins, anthraquinone and glycoside in the leaves of *Acalypha wilkesiana*. It has antifugal and antibacterial properties (Oladunmoye, 2006; Adesina *et al.,* 2000; Ogundiani, 2005). (Hanna *et al.,* 2013) demonstrated that prolonged oral use of *Acalypha wilkesana* at high dose may be toxic.

2.5.2 Phytochemicals

The samples (ethanol extract, aqueous extract and dried powder) of *Acalypha wilkesiana* leaves were analyzed for the presence of alkaloids, saponins, tannins, cardiac glycosides, anthraquinones, steroids, flavonoids, phlobatanins, terpenoids, phytosterols, phenols and oxalate,

according to standard methods.

2.5.3 Uses of *Acalypha wilkesiana*

In recent years, there has been a considerable renewal of interest in the use of medicinal plants in developing countries because herbal medicines have been reported so be safe, easily accessible and without any adverse side effect especially when compared with orthodox drugs. *Acalypha wilkesiana*, a popular medicinal plant has been used by herbal doctors to treat PV, a common superficial mycosis that is associated with cosmetic disfigurement and reduced quality of life.

The Leaf poultices have been used for headache, swelling, cold and wound dressing. Chopped pieces of the dried stem and root in past studies were steeped in alcohol and used for stomach ache and as worm expellant in man in the Delta region of Nigeria (Onocha and Olusanya, 2010). The leaves of this plant are eaten as vegetables in the management of hypertension, in Southern Nigeria (Iwu, 1993). To treat laryngitis and other throat illnesses, the juice is consumed. On the Gazelle Peninsula, leaf juice is consumed with water to treat diarrhoea and dysentery, whereas in New Britain, leaves are used to treat diarrhea. Boiling leaves are applied to the bodies of feverish individuals in the Central Province. Extracts of the leaves of *Acalypha wilkesiana* (Macrophylla) have been shown to possess a wide range of antibacterial and antifungal activity (Oladunmoye, 2006; Alade and Irobi, 1993; Adeshina *et al.*, 2010). Oyelami *et al* (2003) evaluated the efficacy and safety of *Acalypha wilkesiana* ointment in superficial fungal skin diseases. Their formulation produced total inhibition of the growth of *Tinea pedis*, *Pityriasis versicolor* and *Candida intetrigo*. A comparative antimicrobial study on two varieties of *Acalypha wilkesiana* (Macrophylla and Hoffmanni) showed that it possessed a broad spectrum of activity on both bacteria and fungi (Oladunmoye, 2006). To control menstruation, the leaves are crushed, diluted with water, and then consumed. Gastritis and lymphoid swellings are treated with a leaf decoction. In a study by Jekayinfa *et al* (1997), the aqueous extract of *Acalypha wilkesiana* (Macrophylla) showed significant antibacterial and antifungal properties *in vitro* and was found to be reasonably useful in the treatment of eczema.

2.6.1 Lipid Profile

A blood test called a lipid panel or lipid profile counts the number of certain fat molecules called lipids present in the blood. The panel typically includes a test of one's triglycerides as well as four separate cholesterol measures. Cholesterol plays a major role in human heart health. Cholesterol can be both good and bad. High-density lipoprotein (HDL) is good cholesterol and low-density lipoprotein (LDL) is bad cholesterol. High cholesterol in serum is a leading risk factor for human cardiovascular disease such as coronary heart disease and stroke (Tabas, 2002).

A lipid profile report typically include (*Lee* and Siddiqui 2023)

- Total cholesterol
- Low-density lipoprotein (LDL) cholesterol
- Very low-density lipoprotein (VLDL) cholesterol
- High-density lipoprotein (HDL) cholesterol
- Triglycerides

2.6.2 Relevance of Lipid Profile test

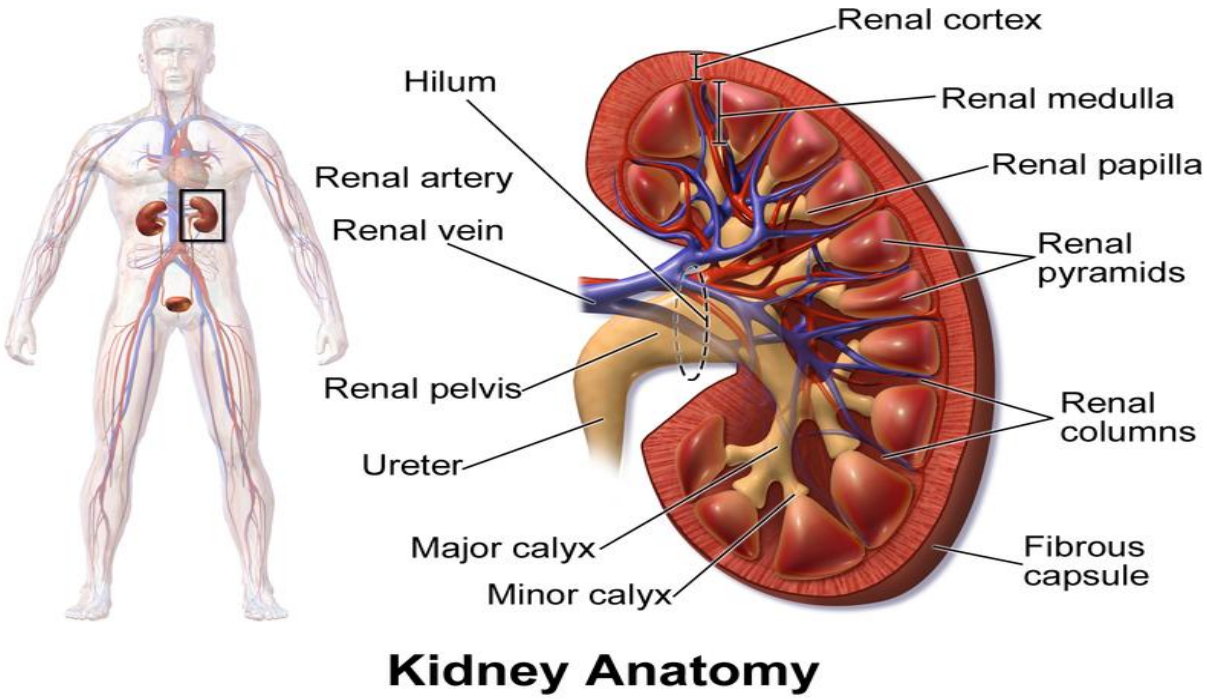
- It is to keep track of how your body is responding to drugs for cholesterol or dietary modifications.

To assist in identifying further medical conditions, such as cardiovascular problems.

2.6.3 THE KIDNEY

The kidneys are two reddish-brown bean-shaped blood-filtering organs. They are located on the left and right in the retroperitoneal space, and in adult humans are about 12 centimeters (4+1⁄2 inches) in length (Mescher, 2016).

Blood enters them through the paired renal arteries, and it leaves through the paired renal veins. A ureter, a tube that transports expelled urine to the bladder, is connected to each kidney. The kidney takes involved in the regulation of toxin elimination, fluid osmolality, acid-base balance, various electrolyte concentrations, and volume of various body fluids. One-fifth of the blood volume that enters the kidneys is filtered in the glomerulus, where filtering takes place. Amino acids, salt, bicarbonate, glucose, and solute-free water are a few examples of compounds that are reabsorbed. Hydrogen, ammonium, potassium, and uric acid are a few examples of chemicals that are released. The structural and operational component of the kidney is the nephron. Interestingly, nephron number varies widely (0.3 to 1.3 million nephrons per kidney) among normal humans (Clark and Bertram 1999).



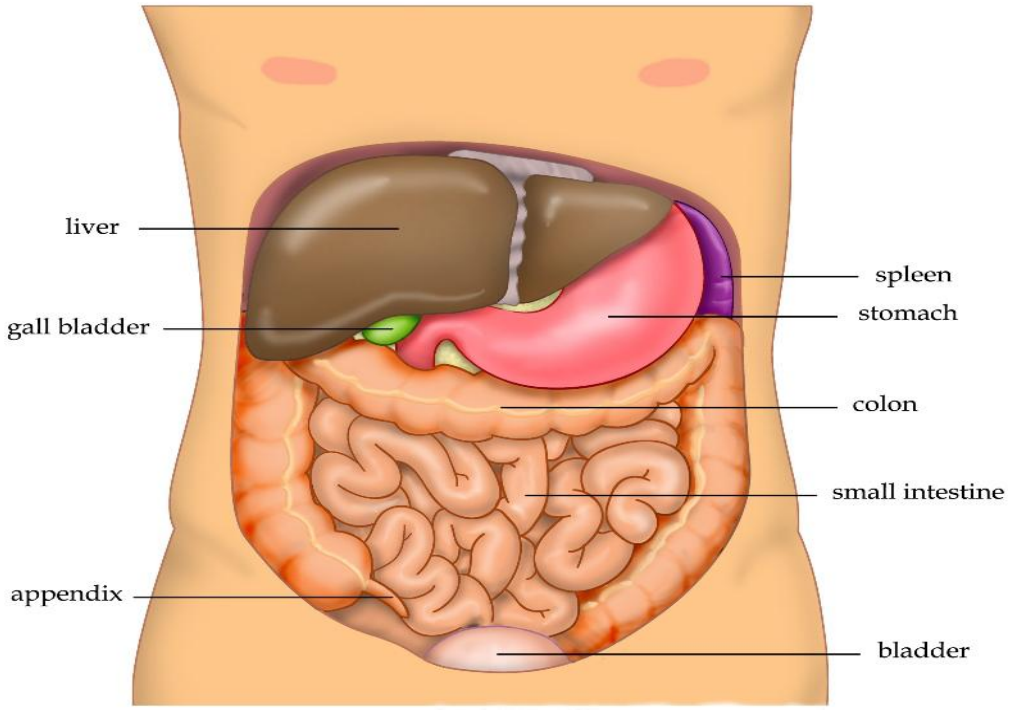
Blausen.com staff (2014)

.Figure 1. 2 Diagram of a kidney

2.6.4 The liver

Only found in vertebrate mammals, the liver is a key metabolic organ that carries out a variety of crucial biological tasks like detoxifying the body and synthesizing proteins and biochemicals required for growth and digesting (Abdel-Misih *et al.,* 2010). It is situated in the right upper quadrant of the abdomen in humans, below the diaphragm, and is primarily protected by the lower right rib cage. In addition to these metabolic functions, it also produces hormones, converts and stores resources like glucose and glycogen, and breaks down red blood cells (Tortora *et al.,* 2008).

Bile, an alkaline fluid containing cholesterol and bile acids that emulsifies and helps the digestion of dietary fat, is produced by the liver, another auxiliary digestive organ. The liver produces bile, which is later concentrated in the gallbladder and discharged to the duodenum to aid in digestion. The gallbladder is a little hollow pouch that lies directly beneath the right lobe of the liver (*"Anatomy and physiology of the liver – Canadian Cancer Society"* 2015). A large number of high-volume biochemical events, such as the creation and breakdown of tiny and complex organic compounds, are regulated by the highly specialized tissue found in the liver, which is primarily made up of hepatocytes. Many of these reactions are required for proper essential activities (Maton *et al.,* 1993).



Wikipidia, 2010.

Figure 1. 3 Diagram of a liver.

2.6.5 Hepatic and Renal function tests (RFT)

A blood test called a hepatic function panel aids medical professionals in examining the liver for damage, infection, or disease. Additionally, it can assess for drug-related liver adverse effects.

A series of examinations known as renal function tests (RFT) are used to assess the function of the kidneys and the entire renal system. To assess the kidneys' present state of health, tests examine levels of numerous chemicals in the blood, including multiple minerals, electrolytes, and proteins. The individual tests included in a kidney function panel can vary by laboratory, but the tests typically performed include:

1. Electrolytes are substance that dissociate in charged particles (ion) in solution and acquire the capacity to conduct electricity (Aronson *et al.,* 2009). They also assist in controlling the body's fluid balance and preserving the acid-base equilibrilium. Electrolytes consist of:
    - Sodium
    - Potassium
    - Chloride

2. Minerals

- Phosphorus – a mineral that is vital for energy production, muscle and nerve function, and bone growth; it also plays an important role as a buffer, helping to maintain the body's acid-base balance.
- Calcium – one of the most significant minerals in the body, required for blood clotting, bone formation, and the proper operation of muscles, neurons, and the heart.

3. Protein

- Albumin – a protein that makes up about 60% of the protein in blood and serves many functions, as to transport drugs throughout the body (Wiglusz and Trynda-Lemiesz 2014) and help metabolism (Yang *et al.,* 2017) . Albumin has been used to transport bilirubin, fatty acids, ions, hormones, and minerals throughout the body through blood circulation (Rahmani-Kukiae *et al.,* 2020).

- Aspartate Aminotransferase – AST (aspartate aminotransferase) is an enzyme that is found mostly in the liver, but it's also in muscles and other organs in your body. When cells that contain AST are damaged, they release the AST into your blood. An AST blood test measures the amount of AST in your blood. Elevated AST levels are commonly related to inflammatory liver disease (viral hepatitis), alcoholic liver disease, cirrhosis, cholestatic syndromes, drug toxicity, acute myocardial infarction, septic shock and skeletal muscle injury/trauma. However, a poor correlation between liver cell damage and plasma amino transferases has been shown (Pratt and Kaplan 2000).
- Alkaline Phosphatase - An enzyme called ALP is present in your bloodstream. Depending on where it comes from, it exists in many forms and aids in the body's breakdown of proteins. ALP is primarily produced in your liver, while some is also produced in your bones, intestines, pancreas, and kidneys. The level of alkaline phosphatase in the blood is checked through the ALP test, which is often part of routine blood tests. The levels of this enzyme in the blood depend on factors such as age, sex, or blood type *(*Lowe *et al*., 2022). Additionally, abnormal levels of alkaline phosphatase in the blood could indicate issues relating to the liver, gall bladder or bones. Kidney tumors and infections as well as malnutrition have also shown abnormal level of alkaline phosphatase in blood *(*Lowe *et al*., 2022).

- Total Bilirubin - Bilirubin is a yellowish substance that your body naturally produces as it breaks down old red blood cells. Bilirubin is found in bile, a fluid your liver produces that aids in food digestion. If your liver is healthy, it will remove the majority of the bilirubin from your body. A bilirubin blood test is used to check the health of your liver. (https://medlineplus.gov/lab-tests/bilirubin-blood-test/)

- Direct Bilirubin -The liver converts bilirubin into a form that our bodies can eliminate. This is referred to as direct or conjugated bilirubin. The small intestine receives this bilirubin as it leaves the liver. A minuscule amount enters our kidney and is eliminated in urine. It is responsible for the yellow color of healing bruises and the yellow discoloration in jaundice. Its breakdown products, such as stercobilin, cause the brown color of feces. A different breakdown product, urobilin, is the main component of the straw-yellow color in urine (Chew *et al.,* 2011).

4. Waste products

- Urea – The waste product urea, which contains nitrogen and is produced when proteins are broken down, is released into the blood by the liver and transported to the kidneys where it is removed from the blood and discharged in the urine. The urea cycle converts highly toxic ammonia to urea for excretion (Cox, 2013).
- Creatinine – another waste product that is produced by the body's muscles; almost all creatinine is eliminated by the kidneys. Diagnostic serum creatinine studies are used to determine renal function (Lewis *et al.,* 2016).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1.1 EQUIPMENT/APPARATUS

Beakers {Pyrex (England)}, Pipette {Pyrex (England)}, Automated micropipette (Micropet and Accumax PRO), Filter Paper {Whatman (England)}, Conical flask {Pyrex (England)}, Measuring cylinders {Pyrex (England)}, Cuvette (Uniben Medical Biochemistry Department), Animal Cages (Uniben Medical Biochemistry Department), Oro-gastric Gavage (Uniben Medical Biochemistry Department), Crucibles {Pyrex (England)}, Analytical Weighing Balance {Technics (England)}, Water distiller {Metter H-80 (Germany)}, Simple Weighing Balance {B. Bran Sc. Inst. Company (England)}, Spectrophotometer (Adventurer OHAUS AR 1530), Bucket Centrifuge {PG Instruments Limited (UK)},Refrigerator {B. Bran Scientific and Instrument Company (England)}, Volumetric Flask (Uniben Medical Biochemistry Department).

3.1.2 Chemical and Reagents

The chemicals used were of analytical grade.

3.2 Methods

3.2.1 Plant materials

Acalypha wilkesiana leaves were gotten from gardens within and outside Benin City and were authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Benin City and roucher number was generated. The leaves were correctly chosen to remove undesirable elements, dried in the air, then ground into a fine powder and weighed.

3.2.2 Preparation of methanol extract

The pulverized leaves were soaked in methanol (95%) for 72 h (3 days). The mixture was occasionally stirred using a magnetic stirrer to ensure proper mixture of the content. The homogenate was then filtered using a sintered funnel (which is equivalent to four folds of bandage or sheet of cheese cloth). The extract (filtrate) was then concentrated using rotary evaporator and weighed.

3.2.3 Preparation of aqueous extract

The pulverized leaves was soaked in distilled water for 72 h (3 days), and treated as described above for methanol extract. (Omage and Azeke 2014b).

3.3.1 Experimental animals

A total of seventy-five adult male wistar rats weighing between 130g to 250g were used for this study. The experimental rats were obtained from a local breeder within Benin City metropolis. The rats were kept in the animal house of the Department of Biochemistry, University of Benin and maintained on a 12-hour light and dark cycle in clean disinfected cages. They were allowed free access to feed (standard pelleted growers feed from Vital Feed, Benin City, Edo State) and water ad libitum duration of the experiment. Prior to the commencement of the study, the animals were allowed to adjust and adapt to the new environment for a period of one week. The experimental procedures performed on the animals were approved by the Animal Ethics Committee of the school of Basic Medical Sciences, College of Medical Sciences, University of Benin, Nigeria. The use of rats for the study were also according to the Ethical Guidelines Involving Whole Animal Testing of the Animal Ethics Committee, School of Basic Medical Sciences, College of Medical Sciences, University of Benin.

After one week of acclimatization, I then divided the experimental rats into seven groups of three rats each for the phase 1 stage of acute toxicity study. The first three groups of three rats each of the same kind was administered with the aqueous extract of the leaf *Acalypha wilkesiana ,* while the next three groups where given the same dose of the methanol leaf extract of *Acalypha wilkesiana.* The last group was given just feed and water because that group served as the control for the experiment. The different doses given to the experimental animals for the phase one stage are for group one 10mg/kg of body weight of both aqueous and methanol extract was given, for group two, 100mg/kg of body weight was given for both aqueous and methanol extract and the third group was given 1000mg/kg of body weight was given for both aqueous and methanol extract. The fourth group was the control.

For phase two of the acute toxicity study. The first three groups of three rats each of the same kind was administered with the aqueous extract of the leaf *Acalypha wilkesiana ,* while the next three groups where given the same dose of the methanol leaf extract of *Acalypha wilkesiana.* The last group was given just feed and water because that group served as the control for the experiment. The different doses given to the experimental animals for the phase two stage are for group one 1600mg/kg of body weight of both aqueous and methanol extract was given, for group two, 2900mg/kg of body weight was given for both aqueous and methanol extract and the third group was given 5000mg/kg of body weight was given for both aqueous and methanol extract. The fourth group was the control.

For sub- acute toxicity study the experimental rats were then randomized into four groups (Group 1 to Group 4) four (4) rats in Group 1(control), then Fifteen (10) rats in each group, Five to a sub group, that is five rats in Group 2A, five rats in Group 2B,five rats in Group 3A,five rats in Group 3B, five rats in Group 4A and five rats in Group 4B respectively.

All animals were weighed and then grouped into 4 according to their weight ranges. All rats were between the weight ranges of 130g to 250g. They were divided into 4 groups based on their weights. Group 1 rats were between the weight ranges of 130g to 163g and served as the control group for the experiment. Group 2 rats were between the weight ranges of 175g to 245g. Group 3 rats were between the weight ranges of 176g to 212g and group 4 rats were rats between the weight ranges of 173g to 198g. The rats were stained with picric acid at different portions of their body for better identification purposes, for instance in each group, a rat was stained at the head, another at the back, another on the abdomen, one on its leg, another on both legs, then one hand, both hands and another stained at the tail. An average of 4 or 5 rats were placed in each group.

3.3.2 Experimental design (sub-acute toxicity)

Table 3.1 showing the experimental design used for the sub-acute toxicity study

GROUP 1(Control)	4 (Rats)	
	A(Aqueous)	B(methanol)
GROUP 2	5 (Rats)	5 (Rats)
GROUP 3	5 (Rats)	5 (Rats)
GROUP 4	5 (Rats)	5 (Rats)

3.3.3 Animal Feeding Protocol

The rats were given their regular feed and water every morning and evening consistently. Acute and sub-Acute toxicity testing was the aim of the study, therefore no disease condition was induced into the animals. The animals were fasted overnight in preparation for sample collection (sacrifice).

3.4.1. Preparation and Dosage Regimen of *Acalypha wilkesiana* extracts

Doses were calculated and prepared daily before administration in $DMSO_2$.

3.4.2. Acute Toxicity Using Lorke's Method

The method of Lorke for acute and sub-acute toxicity was employed in the study, while the LD_{50} was also determined. Acute toxicity according to Lorke, is divided into 2 phases. Phase 1 and phase 2, with each phase divided into 3 subsequent groups.

Table 3.2 Experimental design for Acute Toxicity Study

Phases	Dose mg/kg body weight	No. of animals
Phase 1	10	3
	100	3
	1000	3
	10	3
	100	3
	1000	3
	Control	3
Phase 2	1600	3
	2900	3
	5000	3
	1600	3
	2900	3
	5000	3
	control	3

Phase 1

According to Lorke, group 4 rats under phase 1 served as the control group which wouldn't be given any of the plant extract, then the group 1 rats was given 10mg of plant extract per kilogram of body weight, the group 2 rats was given 100mg of plant extract per kilogram of body weight and the group 3 rats was given 1000mg of plant extract per kilogram of body weight.

For the morning of the acute toxicity testing the rats were starved the evening before, till the morning of collection of sample. After administration of the plant extract to each rat, apart from the control groups, the rats were monitored every hour for any signs of behavioral changes and mortality for the first 4 hours, observations were observed and recorded, afterwards the rats were observed every four hours for the remaining period of the 24 hours. The entire acute toxicity spanned a total of 24 hours after the administration of both plant extracts that morning. According to Lorke, for each group, 3 rats should be used, for each groups, for acute toxicity a total of seven groups for phase 1 making it a total of 21 rats used for the phase 1 stage of the experiment.

Phase 2:

In phase 2, the group 4 rats serve as control as well, that was not given any plant extract, while the group 1 animals were given 1600mg of plant extract per kilogram of body weight, the group 2 animals were given 2900mg of plant extract per kilogram of body weight and the group 3 animals were given 5000mg of plant extract per kilogram of body weight. Reason for dosage per kilogram was because all the rats were weighed before the onset of the experiment, via Lorke's postulates, calculation for the exact dosage of plant extract that each rat was supposed to take.

For example,

Phase 1 Experiment Calculations

Group 1

Rat 1 (Head) – weight (214.5g)

Lorke's formula:

1000mg of extract = 1000g of body weight of rat

∴ Xmg of extract 214.5g of Rat 1(Head) = 214.5mg of extract.

The same procedures were followed for all the rats in the various groups of Phase 1 and Phase 2 to get the exact extract to be administered to each rats.

3.4.3. Administration of Extract

Using an electronic balance, a fragment of both aqueous and methanol plant extract, was weighed to get the accurate amount to be dissolved in $DMSO_2$ that would be enough to be administered to the animals in both phase 1 and phase 2.

Using a gavage attached to a 5ml syringe, the extracts were administered with the aid of the lab scientist, orally into each rat according to the specific lorke's values gotten for each rat via their body weight. The gavage goes deep enough into their throat (upper portion of their GIT), that way the rats cannot vomit or spit out the extracts.

Thereafter a 24-hour watch according to Lorke's method was observed. Every hour for the first 4 hours and then every 4 hours for the next 20 hours.

3.4.4 Observations after administration of plant extract

Table 3.3 Showing phase 1 observations from the administration of methanol and aqueous extract for acute toxicity

GROUP	ADMINISTRATION	TIME FRAME(hr)	OBSERVATION
GROUP 1 (Methanol)	10mg/kg	1	No behavioral change was seen in the rats
GROUP 2(Methanol)	100mg/kg	2	No observed physical or psychological changes seen in the rats, In comparison to the control group that weren't given any extracts.
GROUP 3(Methanol)	1000mg/kg	3	No changes observed in the rats in comparison with the control.
GROUP 1 (Aqueous)	10mg/kg	4	Group 1 given methanol extract, the rats looked weaker and passive in comparison with the control rats.
GROUP 2(Aqueous)	100mg/kg	8	Both control and test animals were in a calm state. No abnormal changes seen between the control group and the test groups.
GROUP 3(Aqueous)	1000mg/kg	12	The rats in group 1 given methanol leaf extract, the rat

			labeled back was active and the rat labeled head and abdomen were sluggish. For group 2, given methanol leaf extract the rat labeled abdomen was active while the one labeled back and head were sluggish. For group 3, the rats given aqueous leaf extract the rat labeled abdomen was active and the other two was sluggish. In general there was no abnormal behavior observed in comparison with the rats in the control group.
Control		16	In group1, given methanol extract of the leaf the rat labeled head was restlessly running around and constantly jerking up in a bid to stand on its legs while the other rats in this group were fine. In group 2, of the rat given aqueous leaf extract the rat labeled head and back were running around restlessly while

			the third one was at rest. In group 3, of rats given methanol leaf extract the rat labeled back was running around restlessly while the other rats were at rest. But overall, there were no abnormal physical changes or mortality seen, in comparison with the control animals.
		20	No abnormal changes observed in comparison with the control
		24	No abnormal changes observed in the test rats in comparison with the control rats

Table 3.4 Showing phase 2 observations from the administration of methanol and aqueous extract for acute toxicity

GROUP	ADMINISTRATION	TIMEFRAME(hr)	OBSERVATION
GROUP 1 (Methanol)	1600mg/kg	1	No observed changes in the rats in comparison with the control.
GROUP 2(Methanol)	2900mg/kg	2	No observed changes in the rats in comparison with the control.
GROUP 3(Methanol)	5000mg/kg	3	No observed changes in the rats in comparison with the control.
GROUP 1 (Aqueous)	1600mg/kg	4	No observed changes in the rats in comparison with the control.
GROUP 2(Aqueous)	2900mg/kg	8	Control seems to be more active than phase 2rats, but no abnormal changes observed
GROUP 3(Aqueous)	5000mg/kg	12	Both control and induced rats were in a calm state.
Control		16	Control animals were at rest while the induced rats were more active and responsive to external stimuli.

		20	No observed changes in the rats in comparison with the control.
		24	No observed changes in the rats in comparison with the control.

3.4.5. Sub-acute Toxicity Using Lorke's Method

After carrying out the acute-toxicity study, there was no mortalities or changes occurred in the animals. Using Lorke's method sub- acute toxicity study was carried out for further toxicity study. Phase 1 of the sub-acute toxicity study was carried out. For 28 days, the animals were fed morning and evening, and administered aqueous and methanol extracts of *Acalypha wilkesiana* orally with the aid of a gavage. The dosage regimen for each rat depended on the rat's weight, calculated using the Lorke's method. The rats were placed in 4 groups and group 1 served as the control group given feed and watered with no extract while group 2 were given 1000mg of both aqueous and methanol extract of the plant extract per kilogram of body weight. The group 3 animals were given 1500mg of both aqueous and methanol extract of the plant per kilogram of body weight and the group 4 animals were given 2000mg of aqueous and methanol extract of the plant per kilogram of body weight. Each week the rat's weights were taken and recorded individually and because of our differentiation mechanism using picric acid, we could easily identify all rats. Lorke's formula was used to calculate the dosage each rat will therefore take for the week based on their weight, so the aqueous and methanol plant extract for the week was prepared for all the experimental animals. The extracts were given to the experimental animals

every morning for 28 days. To mark the end of our sub-acute toxicity study, experimental animals were then sacrificed to begin our biochemical analysis.

3.5. Animal Sacrificing

3.5.1. Procedure

3 animals were brought out from each group to be sacrificed. 3 animals from group 1 which is the control group and 3 animals from group 2, 3 and 4 which were the test groups

The animals were put to death using cotton wool soaked in chloroform and placed in an airtight container, with the rat placed inside the container to suffocate the animal.

After a few minutes of inhalation, the animal dies. The animal was brought out and cut open. Using a 5ml syringe, as much blood as could be found was withdrew from their heart and any other vital organs.

The heart, kidney and liver, was harvested, weighed then placed into disposable packets to run biochemical assays, the samples were placed immediately in freezer to preserve the samples.

3.5.2. Tissue sample collection

After weighing, the harvested organs (hearts, liver and kidneys) they were placed in small plastic bags and put in a freezer before homogenizing the organs and biochemical assays was carried out on them to test for the possible sub-acute toxicity effect of the aqueous and methanol plant extract to evaluate liver function test parameters on the liver of the rats, kidney function test was also carried, electrolyte and lipid profile assay.

3.5.3. Sample Labeling

For easy identification purposes, to know which organs or blood belonged to which rat, I employed specific labeling methods. For instance, “P1-3B-BL”

The P1 served as an indicator that this was phase 1 experiment.

CHAPTER FOUR

4.0 RESULTS

4.1 Acute Toxicity Study of the Extract of *Acalypha wilkesiana*

The median lethal dose (LD_{50}) value for both aqueous and methanol extract was greater than 5,000 mg/kg body weight (Table 4.1). There were no visible signs of toxicity such as shivering, lacrimation, hair erection, heartbeat, reduced feeding, water intake, breathing disorder, disoriented movement, or death observed in all the animals administered the extracts.

Table 4.1 Acute Toxicity Study of the Extract of *Acalypha wilkesiana*

Phases	Dose mg/kg body weight	No. of animals	Mortality
Phase 1	10	3	0
	100	3	0
	1000	3	0
	10	3	0
	100	3	0
	1000	3	0
	Control	3	
Phase 2	1600	3	0
	2900	3	0
	5000	3	0
	1600	3	0
	2900	3	0
	5000	3	0
	Control	3	

Key: (0/39) 0 = number of deaths, 39 = number of rats used.

4.2 Effect of the Methanol Extract of *Acalypha wilkesiana* on the Liver Function Indices of the Rats.

In Table 4.2, it was observed that the activity of aspartate aminotransferase (AST) was increased following the administration of graded doses of the extract when compared with normal control though not with a significant difference ($p > 0.05$). In the same vein, the activity of alanine aminotransferase (ALP) was not significantly impacted by the graded dose of the extract relative to the normal control ($p > 0.05$). Furthermore, the concentration of total bilirubin remained significantly unaffected sequel to the administration of the extract when compared with the normal control ($p > 0.05$). However, the concentration of direct bilirubin and albumin was significantly ($p < 0.05$) elevated in a dose-dependent manner in groups exposed to the extract relative to the normal control when compared to the control.

Table 4.2 Effect of the methanol extract of *Acalypha wilkesiana* on the liver function indices of male Wistar rats.

GROUPS	ALP (U/L)	AST (U/L) x10	T.BIL (mg/dl)	D.BIL (mg/dl)	ALB (mg/dL)
CONTROL	0.026 ± 0.00	35.842 ± 1.46	2.977 ± 0.47	4.229 ± 0.70	1.865 ± 0.05
P12A	0.032 ± 0.00	42.561 ± 1.60	2.815 ± 0.06	0.830 ± 0.05^{a,b}	2.771 ± 0.04^{a,b}
P13A	0.034 ± 0.00	40.614 ± 2.09	2.146 ± 0.17	0.854 ± 0.09^{a,b}	2.777 ± 0.01^{a,b}
P14A	0.025 ± 0.00	39.421 ± 2.20	3.578 ± 0.64	1.022 ± 0.19^{a}	3.546 ± 0.42^{a}

Key: P12A = 1000mg/Kg methanol extract of *A. wilkesiana*; P13A = 1500mg/Kg methanol extract of *A. wilkesiana*; P14A = 2000mg/Kg methanol extract of *A. wilkesiana*; AST = aspartate aminotransferase; ALP = alkaline phosphatase; T.BIL = total bilirubin; D.BIL = direct bilirubin; ALB = Albumin. Values are represented as mean ± SEM. Values in the same column with different alphabets differ significantly ($p < 0.05$).

4.3 Effect of the Aqueous Extract of *Acalypha wilkesiana* on the Liver Function Indices of the Male Wistar rats.

The administration of the graded dose of the aqueous leaf extract of *A. wilkesiana* did not significantly increase the activity of AST when compared with normal control ($p > 0.05$). Similarly, the ALP activity was not impacted by the graded dose of the extract relative to the normal control ($p > 0.05$). In addition, the concentration of total bilirubin remained significantly unaffected in the administration of the extract when compared with the normal control ($p > 0.05$). However, the concentration of direct bilirubin and albumin was significantly ($p < 0.05$) elevated in a dose-dependent manner in groups exposed to the extract relative to the normal control when compared to the control. This result is shown in Table 4.3.

Table 4.3 Effect of the Aqueous extract of *Acalypha wilkesiana* on the liver function indices of male Wistar rats.

GROUPS	ALP (U/L)	AST (U/L) x10	T.BIL (mg/dl)	D.BIL (mg/dl)	ALB (mg/dL)
CONTROL	0.026 ± 0.00	35.842 ± 1.46	2.977 ± 0.47	4.229 ± 0.70	1.865 ± 0.05
P12B	0.034 ± 0.00	37.509 ± 4.34	1.678 ± 0.58	0.744 ± 0.00	2.771 ± 0.13
P13B	0.029 ± 0.00	42.421 ± 1.41	3.586 ± 0.46	0.883 ± 0.06	2.867 ± 0.07
P14B	0.033 ± 0.00	43.789 ± 4.19	4.009 ± 0.72	0.931 ± 0.02	2.854 ± 0.34

Key: P12B = 1000mg/Kg aqueous extract of *A. wilkesiana*; P13B = 1500mg/Kg aqueous extract of *A. wilkesiana*; P14B = 2000mg/Kg aqueous extract of *A. wilkesiana*; AST = aspartate aminotransferase; ALP = alkaline phosphatase; T.BIL = total bilirubin; D.BIL = direct bilirubin; ALB = Albumin. Values are represented as mean ± SEM. Values in the same column with different alphabets differ significantly ($p < 0.05$).

4.4 Effect of methanol extract of *Acalypha wilkesiana* on the Electrolyte Indices of the Male Wistar rats.

Table 4.4 shows the sub-acute effect of the methanol extract of *A. wilkesiana* on the Electrolyte levels in male rats. Results obtained showed that administration of the extract did not significantly impact the levels of plasma electrolyte levels when compared to the normal control ($p > 0.05$). However, at the dosage of 1000 mg extract / Kg body weight of rat, the concentration of plasma sodium ion significantly reduced relative to the normal control ($p < 0.05$). Furthermore, at the highest dose of 2000 mg extract/ Kg body weight of rat, there was a significant decrease in the concentration of potassium relative to the normal control ($p < 0.05$).

Table 4.4 Effect of the Methanol Extract of *Acalypha wilkesiana* on the Electrolyte Indices of Male Wistar rats.

GROUPS	Na^+ (mEq/L) x10	K^+ (mEq/L)	Cl^- (mEq/L)
CONTROL	43.17 ± 9.64	4.23 ± 0.11	50.11± 8.16
P12A	37.31 ± 36.41[a]	4.92 ± 0.04	52.90 ± 0.85
P13A	43.51 ± 16.84	4.80 ± 0.10	35.95 ± 6.86
P14A	43.96 ± 70.94	4.05 ± 0.23	55.95 ± 6.21

Key: P12A = 1000mg/Kg Methanol extract of *A. wilkesiana*; P13A = 1500mg/Kg Methanol extract of *A. wilkesiana*; P14A = 2000mg/Kg Methanol extract of *A. wilkesiana*; Na^+ = sodium; K^+ = potassium; Cl^- = chloride. Values in the same column with different alphabets differ significantly ($p < 0.05$).

Table 4.5 Effect of Aqueous Extract of *Acalypha wilkesiana* on the Electrolyte Levels of the Male Wistar rats.

Graded dose administration of the aqueous extract of *A. wilkesiana* did not significantly increase or decrease the concentration of plasma electrolyte levels in the rat when compared to the normal control ($p > 0.05$). This result is shown in Table 4.5 below.

Table 4.5 Effect of the Aqueous Extract of *Acalypha wilkesiana* on the Electrolyte Levels of the Male Wistar rats.

GROUPS	Na^+ (mEq/L)[10]	K^+ (mEq/L)	Cl^- (mEq/L)
CONTROL	43.17 ± 0.97	4.24 ± 0.11	50.11± 8.16
P12B	44.70 ± 2.72	3.96 ± 0.19	34.27 ± 7.65
P13B	38.57 ± 1.38	3.90 ± 0.29	53.48 ± 3.89
P14B	40.98 ± 2.20	4.47 ± 0.01	50.97 ± 3.42

Key: P12B = 1000mg/Kg aqueous extract of *A. wilkesiana*; P13B = 1500mg/Kg aqueous extract of *A. wilkesiana*; P14B = 2000mg/Kg aqueous extract of *A. wilkesiana*; Na^+ = sodium; K^+ = potassium; Cl^- = chloride. Values in the same column with different alphabets differ significantly ($p < 0.05$).

4.6 Effect of Methanol Extract of *Acalypha wilkesiana* on the Urea and Creatinine Levels of the Male Wistar rats.

In Table 4.6, an increase in the dose of the extract did not significantly increase or decrease ($p > 0.05$) the concentration of urea and creatinine in all the treated groups when compared to the control.

Table 4.6 Effect of the Methanol Extract of *Acalypha wilkesiana* on the Urea and Creatinine levels of Male Wistar rats.

GROUPS	UREA (mg/dl)	CREATININE (mg/dl)
CONTROL	4.275± 0.37	9.621 ± 0.53
P12A	1.992 ± 0.25	10.490 ± 0.24
P13A	1.996 ± 0.25	10.497 ± 0.18
P14A	3.242 ± 1.61	9.582 ± 0.39

Key: P12A = 1000mg/Kg methanol extract of *A. wilkesiana*; P13A = 1500mg/Kg methanol extract of *A. wilkesiana*; P14A = 2000mg/Kg methanol extract of *A. wilkesiana*. Values in the same column with different alphabets differ significantly ($p < 0.05$).

4.7 Effect of Aqueous Extract of *Acalypha wilkesiana* on the Urea and Creatinine Levels of the Male Wistar rats.

In Table 4.7, an increase in the dose of the extract to 2000 mg/Kg bodyweight led to a significant decrease ($p > 0.05$) in the concentration of urea and creatinine when compared to the control in a dose-dependent manner.

Table 4.7 Effect of the Aqueous Extract of *Acalypha wilkesiana* on the Urea and Creatinine levels of the male rats.

GROUPS	UREA (mg/dl)	CREATININE (mg/dl)
CONTROL	4.275 ± 0.37^{a}	9.621 ± 0.53^{a}
P12B	3.488 ± 0.29^{a}	9.144 ± 0.79^{a}
P13B	2.625 ± 0.84^{b}	9.654 ± 0.54^{b}
P14B	1.596 ± 0.18^{c}	8.974 ± 0.45^{c}

Key: P12B = 1000mg/Kg aqueous extract of *A. wilkesiana*; P13B = 1500mg/Kg aqueous extract of *A. wilkesiana*; P14B = 2000mg/Kg aqueous extract of *A. wilkesiana*. Values are expressed as mean ± SEM. Values in the same column with the superscript 'a' differ significantly from the normal control ($p < 0.05$).

4.8 Effect of Methanol Extract of *Acalypha wilkesiana* on the Lipid Profile of Male Wistar Rats.

In Table 4.8, administration of the extract significantly elevated the concentrations of T. CHOL, and LDL-cholesterol but inversely impacted the concentration of HDL-cholesterol in a dose-dependent manner relative to the control ($p < 0.05$). However, the concentration of TG and VLDL-cholesterol increased, but not with a significant difference ($p > 0.05$).

Table 4.8 Effect of the Methanol Extract of *Acalypha wilkesiana* on Lipid Profile of Male Wistar rats.

GROUPS	T. CHOL (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
CONTROL	31.71 ± 1.74^{a}	28.72 ± 0.61^{a}	40.02 ± 0.64^{a}	15.95 ± 2.29^{a}	5.74 ± 0.09^{a}
P12A	59.67 ± 3.04^{b}	32.94 ± 4.57^{ab}	19.16 ± 0.71^{b}	30.72 ± 6.14^{b}	6.59 ± 0.91^{b}
P13A	70.99 ± 4.30^{c}	35.82 ± 3.61^{ab}	15.76 ± 1.68^{b}	30.64 ± 5.12^{b}	6.74 ± 0.36^{b}
P14A	76.27 ± 8.74^{c}	33.65 ± 1.82^{ab}	17.87 ± 2.64^{b}	48.05 ± 5.42^{c}	7.18 ± 0.70^{c}

Key: P12A = 1000mg/Kg methanol extract of *A. wilkesiana*; P13B = 1500mg/Kg methanol extract of *A. wilkesiana*; P14B = 2000mg/Kg methanol extract of *A. wilkesiana*. T. CHOL = Total cholesterol; TG – triglyceride; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; VLDL-C = very low-density lipoprotein cholesterol. Values are represented as mean ± SEM. Values in the same column with different alphabets differ significantly ($p < 0.05$).

4.9 Effect of Aqueous Extract of *Acalypha wilkesiana* on the Lipid Profile of the Male Wistar Rats.

In Table 4.9, administration of the extract significantly elevated the concentrations of T. CHOL, and LDL-cholesterol but inversely impacted the concentration of HDL-cholesterol in a dose-dependent manner relative to the control ($p < 0.05$). However, the concentration of TG and VLDL-cholesterol increased, but not with a significant difference ($p > 0.05$).

Table 4.9 Effect of the Aqueous Extract of *Acalypha wilkesiana* on Lipid Profile of Male Wistar rats.

GROUPS	T. CHOL (mg/dl)	TG (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
CONTROL	31.71 ± 1.74^{a}	28.72 ± 0.61^{a}	40.02 ± 0.64^{a}	15.95 ± 2.29^{a}	5.74 ± 0.09^{a}
P12B	113.41 ± 0.18^{b}	26.06 ± 0.77^{ab}	11.32 ± 5.09^{b}	65.30 ± 10.37^{b}	5.21 ± 0.15^{a}
P13B	128.69 ± 4.86^{c}	28.17 ± 3.54^{ab}	12.14 ± 3.05^{c}	86.87 ± 5.32^{c}	5.63 ± 0.71^{a}
P14B	150.70 ± 11.33^{c}	37.69 ± 3.54^{a}	10.58 ± 9.84^{c}	102.92 ± 7.57^{d}	7.54 ± 0.71^{b}

Key: P12B = 1000mg/Kg aqueous extract of *A. wilkesiana*; P13B = 1500mg/Kg aqueous extract of *A. wilkesiana*; P14B = 2000mg/Kg aqueous extract of *A. wilkesiana*. T. CHOL = Total cholesterol; TG – triglyceride; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; VLDL-C = very low-density lipoprotein cholesterol. Values are represented as mean ± SEM. Values in the same column with different alphabet differ significantly ($p < 0.05$)

CHAPTER 5

5.0 DISCUSSION

If a plant has a severely harmful effect on essential organs at the reported therapeutic level, it has no chance of being used as a medication candidate (Makoshi *et al.,* 2016). Liu, (2005) stated that what differentiates a remedy from a poison is the dose. Plant-based medicines are safer and more environmentally friendly than synthetic ones because they are made of natural substances and have fewer adverse effects (Sengul, *et al.,* 2009). They are therefore a secure and efficient method of treating infections. Additionally, compared to synthetic pharmaceuticals, they are typically cheaper, making them more available to those who might not otherwise have access to treatment (Hosseinzadeh, *et al.,* 2015). Numerous plants that are said to be utilized in herbal medicine systems have not been the subject of thorough toxicity investigations.

The liver is the primary organ in charge of crucial bodily processes like digestion and molecular compound detoxification (Shahat *et al.,* 2018). The majority of used herbal treatments' chemical composition and safety, however, are still unknown. For the treatment of hypertension, the leaves of *Acalypha wilkesiana* are consumed as vegetables (Ikewuchi, *et al.,* 2008). The therapy of gastrointestinal disorders and fungi infections uses the expressed juice or boiling decoction. On *Acalypha wilkesana* plants, insect and disease issues include aphids, mites, and scales (Edward, 2014). Oladunmoye, (2006) reported the presence of saponins, tannins, anthraquinone and glycoside in the leaves of *Acalypha wilkesiana*. It has antifugal and antibacterial properties (Ogundaini, 2005).

The acute toxicity of *A. wilkesiana* methanol and aqueous leaf extracts showed no mortality nor morbidity signs. Throughout the course of the experiment, not a single moribund animal was

obtained. There were no changes in the functioning of the skin, eyes, hair, and respiratory system. Additionally, there were no behavioral or physiological indicators of toxicity, such as disturbed sleep, seizures, breathing problems, restlessness, or hyperactivity. These results give evidence for the non-toxicity of the methanol and aqueous extracts of *A. wilkesiana* over a period of 24 hours.

Acute toxicity test (LD_{50}) of the bark, leaf and root extracts of *P. americana* indicates its relative safety in vivo. Even at the maximum dose of 6000 mg/kg body weight after giving the various extracts to the regular experimental rats, no symptoms of mortality or behavior were seen. The relative safety of natural goods or therapeutic plants in the biological system is frequently swiftly evaluated using an acute toxicity test (Omage, *et al.,* 2017).

Many cells contain transaminases Aspartate transaminate (AST) and Alanine transaminase (ALT), which are biochemical indicators. ALT is a liver-specific enzyme that is also present in the heart and other tissues. It is frequently used to determine how well the liver is functioning (Okuda *et al.,* 2011). However, their concentrations are lower in the pancreas, kidney, and erythrocytes. AST is an enzyme present largely in the liver, heart, kidney, pancreas, and muscles, raised in tissue damage especially in the heart and liver (Costa-Silva *et al.,*2008). The transaminase in the liver In a patient with some degree of intact liver function, aspartate transaminase (AST) and alanine transaminase (ALT) are helpful biomarkers of liver impairment (Mengel *et al.,* 2005). This typically happens in the blood when necrosis or reduced liver cell permeability occur. Hepatic enzymes known as AST and ALT catalyze the addition of an amine group to other molecules in the liver (Yovo *et al.,* 2019).

Therefore, AST and ALP they are two relevant indicators of hepatotoxicity (Da Silva *et al.,* 2010). For the creation of cellular energy, ALT transforms alanine into pyruvate (Chen *et al*., 2004). ALT testing is done to determine its level, especially when biopolymer is injected into the

body. In general, healthy people have ALT levels that are fairly low. When the liver is sick, such as when there is jaundice or hepatitis, ALT is released into the blood. ALT is therefore thought to be a helpful test for spotting liver disease early on (Lin *et al.,* 2008). ALT is commonly measured clinically as part of liver function tests and is a component of the AST/ALT ratio (Lala *et al.,* 2020). When used in diagnostics, it is almost always measured in international units/liter (IU/L) (Ghouri *et al.,* 2010) or μkat. Although sources differ about particular reference range values for patients, the typical reference range for experimental studies is 0–40 IU/L (Lala *et al.,* 2020). Reference ranges for male ≤ 45 IU/L, for female ≤ 34 IU/L (Marshall 2012). ALT is frequently used as a method of screening for liver disorders since noticeably elevated levels of ALT (SGPT) may imply the existence of other medical issues such viral hepatitis, diabetes, congestive heart failure, liver damage, bile duct issues, infectious mononucleosis, or myopathy. Since ALT is an enzyme that primarily functions in the liver, it is typically more sensitive to variations in activity levels than AST (Sodipo *et al.,* 2009).

AST is a key enzyme in the metabolism of amino acids because it catalyzes the reversible transfer of a -amino group between aspartate and glutamate. Red blood cells, the brain, skeletal muscle, kidneys, liver, heart, and gallbladder all contain AST. Additionally, conditions like myocardial infarction, acute pancreatitis, acute hemolytic anemia, severe burns, acute renal illness, musculoskeletal conditions, and trauma can cause an increase in AST levels. Reference ranges for male 8–40 IU/L, for female 6–34 IU/L. Low AST levels that develop over time are quite uncommon, and they may be caused by vitamin B6 deficiencies in the elderly, alcoholics, or those with liver, renal, or inflammatory diseases (Ueland *et al.,* 2015). Low serum AST activity is observed in uremia patients (Ray *et al.,* 2015).

Bilirubin is a red-orange chemical that originates in the normal catabolic route that breaks down heme in vertebrates. This catabolism is a crucial process in the body's elimination of waste products that arise from the degradation of aging or defective red blood cells (Braunstein, 2019).Ultimately, increased levels of bilirubin may be a sign of some disorders since bilirubin is broken down inside the body and its metabolites are eliminated through bile and urine (Smith and Morton 2010). High levels of total bilirubin give several health benefits even in the absence of liver illness (Sedlak and Snyder 2004).Studies have also revealed that levels of serum bilirubin (SBR) (*Slhd.nsw.gov.au.2009)* are inversely related to risk of certain heart diseases (Novotný and Vítek 2003). Findings from the present study showed that the rats exposed to both aqueous and methanol extract did not show significant control ($p > 0.05$) alterations in the concentration of total bilirubin when compared with the normal control. However, the concentration of direct bilirubin and albumin was significantly ($p < 0.05$) elevated in a dose-dependent manner in groups exposed to the aqueous and methanol extract relative to the normal control when compared to the control.

For methanol and aqueous extract, it was observed that the activity of aspartate aminotransferase (AST) was increased following the administration of graded doses of the extract when compared with normal control though not with a significant ($p > 0.05$) difference. In the same vein, the activity of alanine aminotransferase (ALT) was not significantly ($p > 0.05$) impacted by the graded dose of the extract relative to the normal control.

Another hypothesis is that *A. wilkensiana's* flavonoids are what provide the plant's aqueous extract its hepatoprotective properties (Ikewuchi *et al.,* 2010). Researchers have noted that the

hepatoprotective properties of *A. wilkensiana* leaf extract can repair liver cell damage caused by hepatotoxic substances like carbon tetrachloride (Madziga *et al.,* and Atef 2010).

Therefore it can be deduced that both the aqueous and methanol extract of *A. wilkesiana* did not cause any damage to the liver of the rats. Electrolytes are necessary for basic living processes, including the generation and conductivity of action potentials in the nerves and muscles as well as the maintenance of electrical neutrality in cells. In cellular activity, intermediate metabolism, enzyme activities, and electrical gradients, electrolytes are crucial (Lobo 2004). Significant electrolytes include sodium, potassium and chloride.

We obtain electrolytes from our diet and drinks. Electrolyte imbalance is an irregularity in the concentration of electrolytes in the body, and these electrolytes might be out of balance. Electrolyte imbalances can occur when blood levels are either too high or too low, depending on the situation (Hew-Butler *et al.,* 2017). Electrolyte abnormalities frequently result from excessive heat-related dehydration, vomiting, or diarrhea. This is why it's important to replace any lost fluids when it's hot outside or you're feeling under the weather (Tello and Perez-Freytes 2017).

The elemental analysis of the *A. wilkesiana* aqueous extract revealed the presence of macro and micronutrients. The fluid balance of the body is regulated by macronutrients like sodium, potassium, and calcium, which has an impact on cardiac output (Sanni , 2007). In crucial physiological and biochemical processes such neuromuscular excitability, blood coagulation, secretary processes, etc., calcium ions play a role (Sanni, 2007). Despite being the most prevalent electrolyte in the body, calcium is mostly required to create bones (Tintinalli *et al.,* 2016). GI system plays a major role in how it is absorbed and eliminated. The majority of calcium is found extracellularly, and it is essential for the health of nerve cells, muscle cells, enzyme activity, and blood clotting (Tintinalli *et al.,* 2016). When there is an excessive amount of calcium in the

blood, it is known as hypercalcemia. It happens over 10.5 mg/dL (Walls *et al.,* 2018). When blood calcium levels are too low, often less than 8.5 mg/dL, the condition is referred to as hypocalcemia (Walls *et al.,* 2018). Bone mineralization requires the right extracellular fluid and periosteal calcium and phosphate ion concentrations (Robert *et al.,* 2000). Potassium can oxidize substances, function as an astringent, and eliminate organic toxins, particularly alkaloids (Aliu, 2005). The glomerulus in the kidneys is where potassium is filtered. At the thick ascending loop of Henle and the proximal convoluted tubule, potassium is re-absorbable (Gumz *et al.,* 2015). The kidneys are responsible for excreting the majority of potassium from the body (Tintinalli *et al.,* 2016). This means that they play a critical role in preserving a healthy potassium balance in the blood. Hyperkalemia denotes a very high level of potassium in the blood. In patients with renal failure, hyperkalemia is often brought on by decreased kidney excretion, a transfer of potassium into the extracellular space, or increased ingestion of foods high in potassium (Walls *et al.,* 2018).

Undoubtedly one of the most important electrolytes in the extracellular fluid is sodium, an osmotically active cation. It is in charge of preserving the extracellular fluid volume and controlling the cell membrane potential. As part of active transport, sodium and potassium are transferred across cell membranes (Ferrannini, 2017). The kidneys regulate sodium levels. The majority of sodium reabsorption occurs in the proximal tubule. Sodium is reabsorption in the distal convoluted tubule. Aldosterone regulates sodium-chloride symporters, which are responsible for transporting salt (Palmer and Schnermann, 2015). The term hypernatremia refers to a very high level of salt in the blood. The majority of those who suffer from this condition either experience water loss from diarrhea, changed thirst sensations, difficulty to drink water, kidneys' failure to produce concentrated urine, or excessive salt intake (Tintinalli *et al.,* 2016).

Hyponatremia means that the concentration of sodium in the blood is too low (Tintinalli *et al.,* 2016). This relatively common electrolyte abnormality may be a sign of a disease process, but in the medical context, the administration of hypotonic fluids is more frequently to blame (Tintinalli *et al.,* 2016).

The administration of the methanol extract did not significantly impact the levels of plasma sodium electrolyte levels when compared to the normal control ($p > 0.05$). However, at the dosage of 1000 mg extract / Kg body weight of rat, the concentration of plasma sodium ion significantly reduced relative to the normal control ($p > 0.05$).

In the highest dose of 2000 mg extract/ Kg body weight of rat, there was a substantial decline in the concentration of potassium relative to the normal control ($p < 0.05$).

Graded dose of *A. wilkesiana's* aqueous extract was not given because it significantly increased or decreased the concentration of plasma sodium, calcium and potassium electrolyte levels in the rat when compared to the normal control ($p > 0.05$).

Creatinine and urea levels are good indicators of kidney function (Yovo *et al.,* 2019). Serum urea and creatinine levels were used to evaluate renal function. Urea and creatinine are crucial indicators of renal function (El Khasmi and Farh, 2022). These metabolism products have a constant level under normal conditions (Li *et al.,* 2022). Renal impairment is reflected by their decrease or increase (Pritchard *et al.,* 2019). A decrease in serum creatinine had been demonstrated by Pritchard and his associates to be a sign of cachexia. A spike in serum urea levels can indicate nephropathy, dehydration, an electrolyte imbalance, hypoalbuminuria, and tissue catabolism (Pritchard *et al.,* 2019). Because these parameters did not vary significantly in the experimental rats exposed to the aqueous and methanol extracts relative to controls, a normal kidney function was proposed.

The body uses lipids, which are stored as fats and fatty compounds in the blood and tissues, as a source of energy. Lipids have a variety of uses, including energy storage, signaling, and acting as structural elements of cell membranes (Subramaniam *et al.,* 2011). The lipid profile test is a collection of tests done simultaneously to assess any potential risk of coronary heart disease, or to do so as a preventive step, depending on factors including dietary choices, stress levels, physical activity levels, and lifestyle-related factors (https://www.portea.com). Lipids help to maintain the body's metabolism. High cholesterol, specifically high LDL ("bad") Cardiovascular disease is significantly influenced by cholesterol. As a result, high cholesterol can play a key role in life threatening conditions like heart attack and stroke (https://www.healthline.com). In Table 4.9, administration of the extract significantly elevated the concentrations of T. CHOL, and LDL-cholesterol but inversely impacted the concentration of HDL-cholesterol in a dose-dependent manner relative to the control ($p < 0.05$). However, the concentration of TG and VLDL-cholesterol increased, but not significantly ($p > 0.05$).

5.1 CONCLUSION

From the study carried out, the results revealed that it is more likely that when aqueous and methanol are administered extracts of *Acalypha wilkiasiana* are administered, there were no observable toxic effect. However, the sub- acute toxicity study showed mild effect on increase of doses of the extracts which could be as a result of prolonged administration.

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