BIOCHEMICAL EVALUATION OF THE KIDNEY IN MALE RATS FOLLOWING SUB-CHRONIC CONSUMPTION OF METHANOL LEAF EXTRACT OF *Anthocleista grandiflora*

BY

Lilian Osholene IDANWEKHAI (Miss)

LSC1706039

DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN,

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A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR OF SCIENCE DEGREE (B.Sc.) IN SCIENCE LABORATORY TECHNOLOGY (PHARMACOLOGY/PHYSIOLOGY TECHNIQUES)

CERTIFICATION

This is to certify that this project work was carried out by **Lilian Osholene IDANWEKHAI (Miss)** with Matriculation number **LSC1706039**, under the supervision of **Mr. J. O. Oseyomon** in Science Laboratory Technology Department, Faculty of Life Sciences, University of Benin, Benin City, Edo State.

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MR. J. O. OSEYOMON ***Date***

Project Supervisor

_____ _____

Dr. ***Date***

Project Coordinator

_____ _____

Dr. E. O. OSHOMOH ***Date***

Head of Department

DEDICATION

ACKNOWLEDGEMENT

ABSTRACT

Anthocleista grandiflora has a history of traditional herbal use and has been reported to possess a wide range of therapeutic properties. This current study aimed to explore the effect of chronic administration of a methanol seed extract of *Anthocleista grandiflora* on the biochemical indicators of renal function in male rats. For this study, twenty male rats were randomly divided into four groups, each consisting of five rats. The extract was administered orally at doses of 200, 400, and 800 mg/kg of body weight for a duration of 28 days. The kidney was harvested and processed histologically and blood samples were taken for biochemical assays. Statistical analysis was conducted using one-way ANOVA with Graph Pad Prism. The findings revealed that there were no significant differences in the sum of the means of all the parameters measured across the groups ($P>0.05$) when compared with control. Based on the absence of elevated urea and creatinine levels in the blood and the absence of any abnormalities in the histological examination of kidney tissues across all treatment groups, it can be inferred that *Anthocleista grandiflora*, administered at varying doses over a four-week period, does not appear to have any adverse effects on kidney function.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Traditional medicine has a rich history of utilizing plants and their derived products to address infectious diseases. The use of medicinal plants remains significant as therapeutic remedies and still plays an important role in primary health care in developing countries such as Nigeria (Onyegbule *et al*., 2014). For generations, people relied solely on the utilization of flowers, leaves and bark from plants as their primary source of medicinal remedies. Although synthetic drugs have also come into use, in many instances, they are replicas or modifications of chemicals identified in plants (Conway, 1973).

Plants, which constitute a major component of foodstuffs in humans, have formed the basis of various traditional medicine systems and folk medicines that have been practiced for thousands of years during the course of human history (Pan *et al*., 2013). Until now, plants/herbs are still highly esteemed all over the world as a rich source of therapeutic agents for the treatment and prevention of diseases and ailments; at present, more than 35,000 plant species are used for medicinal purposes around the world (Yirga *et al*., 2011). In conventional Western medicine, 50–60% of pharmaceutical commodities contain natural products or are synthesized from them; 10–25% of all prescription drugs contain one or more ingredients derived from plants (Cameron *et al*., 2005).

Plants are presented as a promising source for the search of new substances, because they have a higher molecular diversity when compared to products synthetic chemically (De Assis *et al*.,

2018; Surendra and Roopan, 2016). There is 1–10% out of 250.000–500.000 plant species have been fully studied for their potential medicinal value (Verpoorte, 2000).

There is growing interest in correlating phytochemical constituents of plants with their pharmacological activity (Vukovic *et al*., 2007). Flavonoids have been reported to possess antibacterial activity, in which it has the ability to form complex with extracellular soluble proteins and bacterial cell walls (Tsuchiya *et al*., 1996). In the same manner, purified alkaloids, as well as their synthetic derivatives, are used for their various biological effects such as analgesic, antispasmodic and bactericidal as remedies for diseases (Evans, 2002).

Investigations into the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern synthetic organic chemistry and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents (Roja and Rao, 2002).

Many medicinal plant products are used in Africa for the management of pain and inflammation and their efficacy and potency are traditionally acclaimed (Umar *et al*., 2013). Phytochemicals with various bioactivities, including antioxidant, anti-inflammatory and anticancer properties are extensively extracted from many plant species (Khalifa *et al*., 2013).

Routinely, the kidneys are exposed to high concentrations of medications or their metabolites because their intrinsic function is to metabolize, concentrate, and excrete compounds (Thomson *et al*., 2002). Hence, it's not unexpected that, akin to prescription drugs, numerous dietary supplements have been linked to nephrotoxicity. This nephrotoxicity can result either from a

direct toxic impact or as a secondary consequence of liver impairment, rhabdomyolysis, or the formation of kidney stones.

In addition to the liver, the kidney plays a significant role in metabolizing toxic substances, making it a vital organ in the detoxification process. It receives about 1200 ml blood per minute (Tortora *et al*., 2006) containing a lot of chemical compounds and are at high risk to be exposed to toxic compounds. Consequently, it is plausible that elevated doses of *Anthocleista grandiflora* could potentially induce harm to kidney tissues. This potential damage can be assessed by monitoring the levels of urea and creatinine in the blood, which serve as indicators of kidney injury. Moreover, kidney damage was also assessed through histological examination of kidney tissue.

The kidneys are a pair of bean-shaped organs situated bilaterally on both the left and right sides of the body in vertebrates. These vital organs are positioned toward the rear of the abdominal cavity within a region known as the retroperitoneal space. In adults they are about 11 centimetres (4.3 in) in length (Cotran *et al*., 2005). Blood is supplied to the kidneys through the paired renal arteries, and it exits via the paired renal veins. Additionally, each kidney is connected to a ureter, a tubular structure responsible for transporting excreted urine to the bladder. Within the kidney, the nephron serves as both the structural and functional unit. It's worth noting that an average adult kidney comprises approximately one million nephrons. The nephron utilizes four processes to alter the blood plasma which flows to it: filtration, reabsorption, secretion, and excretion (Clapp, 2009). The kidney actively participates in maintaining the volume of various body fluid compartments, fluid osmolality, acid-base balance, various electrolyte concentrations, and the removal of toxins by one or more of these mechanisms. The glomerulus, where around one-fifth

of the total blood volume entering the kidneys is filtered, is where this filtration process predominantly takes place. Reabsorbed substances include solute-free water, salt, bicarbonate, glucose, and amino acids, to name a few (Emamian *et al*., 1993).

1.2 AIM OF THE STUDY

The aim of this study is to determine the effect of methanol leaf extract of *Anthocleista grandiflora* on the kidney function of rats.

1.3 OBJECTIVES OF THE STUDY

i. To determine the effect of methanol leaf extract of *Anthocleista grandiflora* on the level of urea in the kidney.
ii. To determine the effect of methanol leaf extract of *Anthocleista grandiflora* on the level of sodium in the kidney.
iii. To determine the effect of methanol leaf extract of *Anthocleista grandiflora* on the level of potassium in the kidney.
iv. To determine the effect of methanol leaf extract of *Anthocleista grandiflora* on the level of bicarbonate in the kidney.
v. To determine the effect of methanol leaf extract of *Anthocleista grandiflora* on the level of chloride in the kidney.
vi. To determine the effect of methanol leaf extract of *Anthocleista grandiflora* on the level of creatinine in the kidney.

CHAPTER TWO

LITERATURE REVIEW

2.1 HERBAL MEDICINE

Medicinal plants are the richest bio-resource of drugs for traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Handa *et al*., 2008). In underdeveloped nations like Nigeria, the use of medicinal plants as therapeutic medicines is still significant and plays a vital role in primary healthcare.

Plants have an almost limitless ability to synthesize aromatic substances, most of which are phenols or their oxygen-substituted derivatives (Gautam *et al*., 1993). Most are secondary metabolites, of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total (Schultes, 1978). These compounds frequently serve as the plants' natural defence mechanisms, shielding them from herbivores, insects, and microbial predators. Some of these substances, like terpenoids, are involved in the distinctive smells that plants are known for, while others, like quinones and tannins, are in charge of the coloration of the plant. Many compounds are responsible for plant flavor (e.g., the terpenoid capsaicin from chili peppers), and some of the same herbs and spices used by humans to season food yield useful medicinal compounds (Cowan, 1999).

Medicinal plants has been defined by WHO consultative group as any plant which in one or more of its organs contains substances that can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Andrews, 1982).

Traditional medicine has a long history of using plants and items derived from plants to treat infectious diseases. To validate the medicinal claims found in folklore, various screening methods have been employed on different parts of plants to extract bioactive compounds. Traditional medicine practice is widespread throughout the world and it can be described as the total combination of knowledge and practices, whether explicable or not, used in diagnosing, preventing or eliminating a physical, mental or social disease and which may rely exclusively on past experience and observation handed down from generation, verbally or written (Sofowora, 1984).

For a considerable period, people relied solely on the leaves, flowers, and bark of plants as their primary source of medicinal remedies. Although the use of synthetic medications has increased, it's important to note that in many instances, these drugs are either exact duplicates of substances found in plants or chemical alterations of those compounds. In orthodox medicine, a plant may be subjected to several chemical processes before its active ingredient is extracted, refined and made ready for consumption while in traditional medicine a plant is simply eaten raw, cooked or infused in water or native wine or even prepared as food (Conway, 1973).

Previous study had shown that the herbal medicine is still the mainstay of about 75 to 80% of the world population for the primary healthcare because of better cultural acceptability, better compatibility with the human body and fewer side effects (Cohen, 1992).

According to Elujoba (1997), a plant becomes a medicinal plant only when its biological activity has been ethnobotanically reported or scientifically established. American botanist John Harshburger first used the term "ethnobotany" in 1896. It was first used to refer to the study of plants used by indigenous and prehistoric peoples. The traditional knowledge that indigenous

populations have about the variety of plants in their surroundings and how various groups make use of local native plants has been the definition of ethnobotany from the field's start. Essentially, ethnobotany is the study of the ways that local societies use native plants for things like food, clothing, and medicine.

Historically, plants not only provided man with food but also with means of healing (Aiyeloja and Bello, 2006). Ethnobotany and ethno-medical studies are today, recognised as the most viable methods of identifying new medicinal plants or refocusing on those earlier reported for bioactive constituents (Adjanahoun *et al*., 1991). There is an increasing need to capture the traditional knowledge of plant use from the older generation, which is rapidly diminishing. This is essential to safeguard and uphold the valuable uses of these plants. Moreover, it serves as a foundation for scientific investigations into the pertinent phytochemical components. Such research aims to inform the development of pharmaceutical research and production. The general public should eventually have access to this information, especially in tropical areas where up to 90% of the population relies on medicinal plants for their basic healthcare need.

Plants are a great source of medicines, which are useful in the treatment of various diseases (Bako *et al*., 2005). According to the World Health Organization (WHO 2001b), 80% of the world population use medicinal plants in the treatment of diseases and in African countries, this rate is much higher. It has been estimated that up to 90% of the population in developing countries rely on the use of medicinal plants to help meet their primary health care needs (WHO, 2002).

According to a report by the FAO (Food and Agriculture Organization) in 2000, a minimum of 25% of the drugs included in modern pharmacopoeia have their origins in plants. Additionally,

many other medications are synthetic analogues that have been developed based on prototype compounds initially isolated from plants. Over 50% of all modern clinical drugs are of natural product origin (Stuffness and Douros, 1982) and natural products play an important role in drug development programs of the pharmaceutical industry (Baker *et al*., 1995). Also, Medicinal plants have demonstrated their contribution to the treatment of diseases such as HIV/AIDS, malaria, diabetes, sickle-cell anaemia, mental disorders (Elujoba *et al*., 2005) and microbial infections (Okigbo *et al*., 2005). Plant-derived drugs serve as valuable prototypes for the development of medications that are not only more potent but also less toxic.

As part of its health initiatives, the World Health Organisation (WHO) actively encourages, promotes, and enables the effective use of herbal medicine in poor countries. Despite the knowledge already in existence, higher plants' full potential as a source of novel medications is mostly untapped. Hence, there is an increase in the investigations on plants as a source of new biomolecules for human disease management (Grierson and Afolayan, 1999).

Medicinal uses of plants range from the administration of the roots, barks, stems, leaves and seeds to the use of extracts and decoctions from the plants (Ogbulie *et al*., 2004). Iwu *et al*. (1999) reported that the primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment. Furthermore, the increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of several drugs and chemotherapeutics from these plants as well as from traditionally used rural remedies (UNESCO, 1998). Investigations into the chemical and biological activities of plants during the past two centuries have yielded compounds for the development of modern synthetic organic chemistry

and the emergence of medicinal chemistry as a major route for the discovery of novel and more effective therapeutic agents (Roja and Rao, 2000).

Plants that are consistently found to be effective and are commonly prescribed may contain compounds with the potential to become drug candidates. These plants deserve more research, and it could be fair to suggest that they be thoroughly examined. Due to the importance of medicinal plants to healthcare, scientific studies of them have been started in many nations. The active principles differ from plant to plant due to their biodiversity and they produce a definite physiological action on the human body (Edeoga *et al*., 2006).

In addition, these chemical principles vary in distribution within the plant parts, as well as their occurrence within plant species (Bako *et al*., 2005). These are influenced mainly by cultivation period, season of collection and plant-to-plant variability in the medicinal content (Nalawade and Tsay, 2003), that is why phytochemical screening of plants must be done constantly, even on the ones whose secondary metabolites are already known. Due to expanding focus on the use of traditional medicine, it has become necessary to document the trado-medicinal uses, as well as 4 expand our knowledge of the possible active principles involved in the acclaimed efficacies of plants used in this system

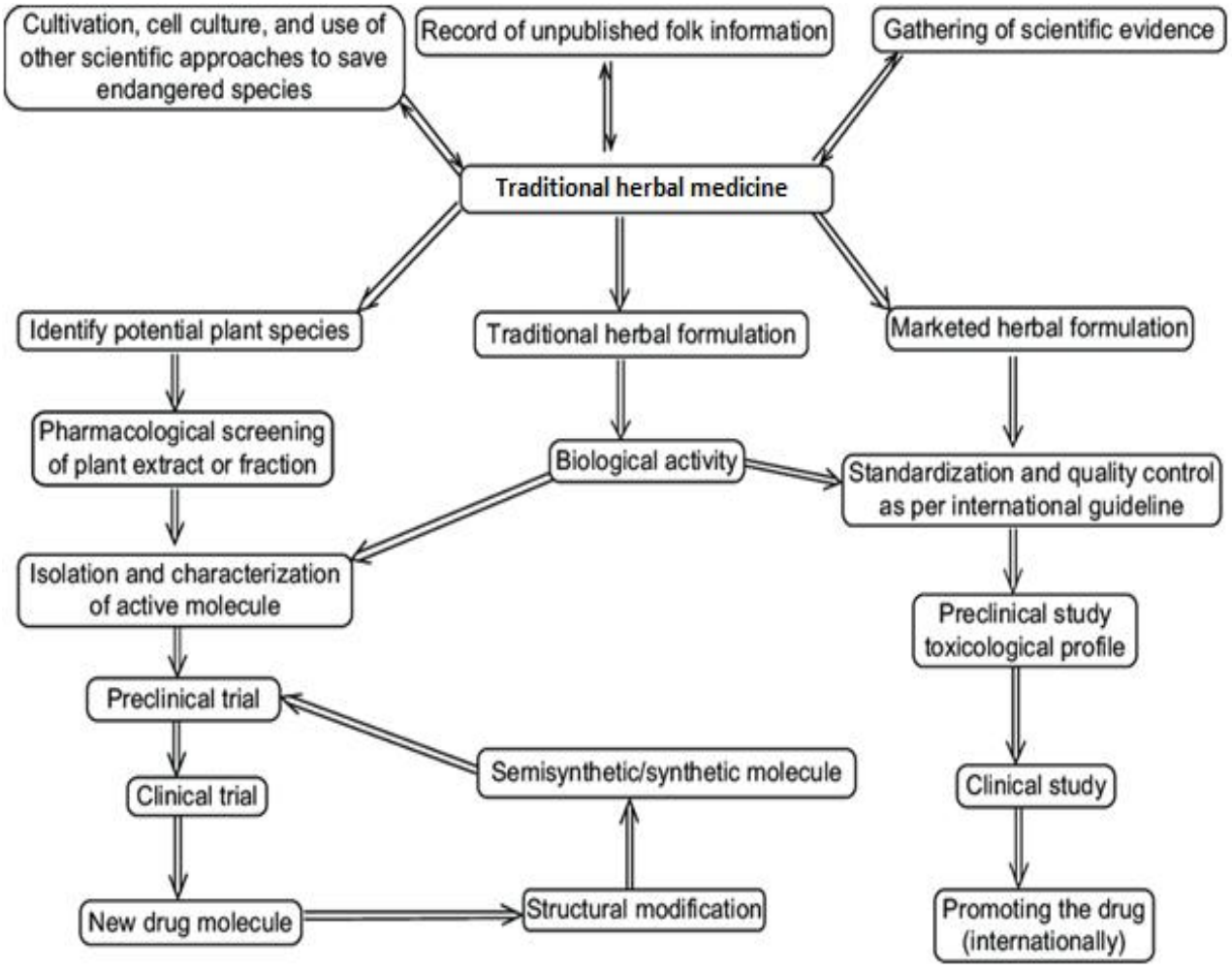

Figure 1: Strategy for advancement and integration of traditional herbal medicine into modern medicine (Sen and Chakraborty, 2015**).**

2.1.1 HERBAL MEDICINE IN AFRICA

One of the oldest and, probably, most diversified treatment systems currently in use is African traditional medicine. The continent of Africa, which is frequently called the "cradle of humanity," is rich in biological and cultural diversity, which is reflected in the diverse regional variances in healing techniques. In all of its manifestations, this traditional medical system takes a holistic approach, addressing both the physical and emotional facets of wellbeing. The traditional healer typically diagnoses and treats the psychological basis of an illness before prescribing medicines, particularly medicinal plants to treat the symptoms (WHO, 2008; Gurib-Fakim *et al*., 2010 Gurib-Fakim and Mahomoodally, 2013). There are two main reasons why traditional medicine continues to be valued within the African healthcare system. The first is that allopathic drugs and Western medical procedures are not widely available. Due to the exorbitant expense of contemporary medical treatment or a lack of medical service providers, a sizeable section of the African population lacks access to it. Second, there is a lack of effective modern medical treatment for some ailments such as malaria and/or HIV/AIDS, which, although global in distribution, disproportionately affect Africa more than other areas in the world (Mahomoodally, 2013).

The most prevalent form of traditional medicine widely practiced throughout the African continent involves the use of medicinal plants. In many regions of Africa, medicinal plants represent the most readily available healthcare resource accessible to local communities. Furthermore, they are often the preferred choice for patients seeking remedies. For most of these people, traditional healers offer information, counseling, and treatment to patients and their families in a personal manner as well as having an understanding of their patient’s environment

(WHO, 2008; Gurib-Fakim and Mahomoodally, 2013). Africa is undeniably endowed with a vast array of biodiversity resources. It is estimated to harbor between 40,000 and 45,000 plant species with significant potential for various applications. Remarkably, approximately 5,000 of these plant species are actively employed for medicinal purposes. This is not surprising since Africa is located within the tropical and subtropical climate and it is a known fact that plants accumulate important secondary metabolites through evolution as a natural means of surviving in a hostile environment (Manach *et al*., 2004). Because of her tropical conditions, Africa has an unfair share of strong ultraviolet rays of the tropical sunlight and numerous pathogenic microbes, including several species of bacteria, fungi, and viruses, suggesting that African plants could accumulate chemopreventive substances more than plants from the northern hemisphere (Mahomoodally, 2013).

The potentials of plant-derived compounds from African medicinal plants have been reported and the interest to use medicinal plants in treatment and management of disease conditions is growing rapidly in Africa even among educated African urban dwellers (Oguntibeju, 2018). The truth is that African traditional medicine is usually the first contact in meeting the primary health care need in Africa and is related to its affordability, accessibility, cultural and spiritual acceptance, and knowledge of its preparations and use (Abdullahi, 2011; Maroyi, 2013;).

Africa is blessed with a variety of plants that can be utilised medicinally, and they have made the most of this. In fact, out of the approximated 6400 plant species used in tropical Africa, more than 4000 are used as medicinal plants (Stanley, 2004). Medicinal plants are extensively employed in the treatment of a wide range of diseases and illnesses, and their uses and effects have gained increasing attention in Western societies. These plants are not only selected for their

therapeutic properties but also frequently hold symbolic and spiritual significance. For example, leaves, seeds, and twigs that are white, black and red are seen as especially symbolic or magical and possess special properties (Helwig, 2005).

Millions of people in Africa depend on traditional healers and treatments produced from local plants for their health. According to the International Development Research Centre (IDRC), one estimate puts the number of Africans who routinely use these services for primary health care as high as 85% in Sub-Saharan Africa (Stanley, 2004).

Before the establishment of science-based medicine, traditional medicine was the dominant medical system for millions of people in Africa but the arrival of the Europeans was a noticeable turning point in the history of this ancient tradition and culture (Abdullahi, 2011). While contemporary scientific medicine has been successful in affluent nations, its effects have not been as good in many underdeveloped African countries. Western healthcare practices can make a difference in certain aspects of healthcare, such as combating the spread of diseases. However, they often struggle to fully integrate into the local culture and society, as noted by Conserve Africa in 2002. This underscores the essential role that traditional African practitioners play in the healthcare system, particularly in areas where Western medicine may not fully align with the local culture and practices.

The therapeutic potential of traditional medicine in the African environment is great and requires further in-depth study to improve methods and training and to form a more effective organization within the ranks of traditional healers (Tella, 1979).

2.3 Anthocleista grandiflora

The Anthocleista grandiflora is a plant that can grow as both trees and shrubs. It currently belongs to the Gentianaceae family, having previously been classified in the Loganiaceae family. It falls under the broader category of Angiosperms. The genus Anthocleista contains about 14 species distributed in tropical Africa, in Madagascar and on the Comoros (Ateufack et al., 2007; Backlund et al., 2000). Although Gbolade (2012) mentioned that the Anthocleista genus comprised 15 species, there are currently 14 recognized species in the Anthocleista genus. These include: *Anthocleista madagascariensis* Baker, *Anthocleista longifolia (Lam.) Boiteau, Anthocleista djalonensis A. Chev, Anthocleista vogelii Planch, Anthocleista obanensis Wernham, Anthocleista procera Lepr. ex Bureau, Anthocleista amplexicaulis Baker, Anthocleista scandens Hook f.,* and *Anthocleista nobilis G* (De Wilde, 2011).

Anthocleista grandiflora is also known as cabbage tree in English language. This is because the stem of some species are unbranched or branched only at the top with huge leaves clustered at the end of the shoot (Edwin-Wosu *et al.,* 2015). A large proportion of the names used to describe *Anthocleista grandiflora* seemed to be within the areas or regions of the country in which they are found such as, the Hausas (Northern Nigeria) calls it 'Kwari' (quiver), the Yoruba's (Western Nigeria) call it Apa oro' or sapo and the Ibos (Eastern Nigeria) call it 'Mpoto' (Hyde, *et al.,* 2015). Anthocleista grandiflora has a history of traditional medicinal applications, including the treatment of conditions such as fever, wounds, inflammatory diseases, stomach ache, constipation, and diabetes, among others. The phytochemical, morphological and molecular data of Anthocleista supports its transfer to the Gentianaceae (Jensen and Schripsema, 2002). As a result, it is appropriate to classify the genus Anthocleista as belonging to the family Gentianaceae of the order Gentianales.

Taxonomy of *Anthocleista grandiflora* Gilg

Kingdom: Plantea

Phylum: Tracheophyta

Class: Mannoliopsida

Order: Gentianales

Family: Gentianaceae

Genus: Anthocleista

Specie: *Anthocleista grandiflora*

2.3.1 Botanical Description

In general, *Anthocleista grandiflora* is a genus characterized by trees that can reach heights of 6 to 20 meters or even taller, with trunks measuring 15 to 55 centimeters in diameter. The twigs of these trees often have spines. The leaves are arranged oppositely and are exceptionally large, reaching dimensions of up to 150 square centimeters, with short petioles or being sessile. The upper surface of the leaf typically exhibits a dark green color and may possess a shiny or glossy appearance. The inflorescence is terminal, often dichasial or branching in other ways, and is notably large. The sepals, which number 4, are circular or wider than they are long. Corolla white (or creamy), actinomorphic, thick, fleshy; lobes 8–16, contorted in bud; ovary is superior and fruit a berry (Adongo *et al.,* 2012; Ajibesin *et al.,* 2008). *Anthocleista grandiflora* are present in tropical Africa, from Sierra Leone in the west to Uganda in the north and to Angola in the south (Alade *et al.,* 2011) including Zambia, and Kenya in the East. *Anthocleista grandiflora* is typically located in lowland forests and aquatic ecosystems. These are perennial trees that favor

tropical climates and are commonly found in lowland secondary rainforests. *Anthocleista grandiflora* is known to thrive as a common seral plant on abandoned farmland in the tropical African forest regions. *A. grandiflora* are riparian in habitat (Ariwaodo *et al*., 2012). *Anthocleista grandiflora* is not a cultivated plant, hence there is little information available regarding its cultivation.

2.3.2 Ethnomedicinal uses

A significant body of literature exists that documents the extensive utilization of *Anthocleista grandiflora* in traditional medicine for addressing a wide range of health conditions. They are commonly used for the treatment of constipation, malaria fever, typhoid fever, hypertension, stomach aches, hemorrhoids, syphilis, diabetes, and used as a contraceptive, laxative and purgative (Borokini *et al.,* 2013). The use of the bark and root as purgative and antidote for snake bite; the bark-sap for ear and eye treatments; and the bark and root in the healing of dropsy, swellings, oedema, gout and venereal diseases have been documented (Dibong *et al.,* 2011). The root of the plant is commonly prepared in combination with potash and used to treat fungal skin infections as well as infections caused by filarial worms, including loa-loa filariasis. Additionally, a decoction made from the root is ingested to address hernias in the groin area. Applied externally, it is used as a wash or bath, or as a vapour bath, to treat a range of conditions including leprosy, venereal diseases, acute inflammations, boil on the skin, oedema and scrotal elephantiasis (Lawal *et al.,* 2010). A tea made from finely chopped soft root bark, steeped in water, is ingested to alleviate thrush. Infusions made from the bark are employed to treat fractures in women's bones. An extract obtained from the bark of the twig is employed as eye

drops and as a remedy for relieving diarrhea in infants. Powdered stem bark, when mixed with the roots of *Aloe buettneri*, is consumed to treat hepatitis, jaundice, and cirrhosis. Additionally, a tincture prepared from the leaves is ingested to address cases of diarrhea and dysentery. A decoction of dry, fallen leaves is drunk to treat jaundice (Malan *et al.,* 2015)

2.3.3 Chemical constituents

Previous studies on *Anthocleista grandiflora* showed the presence of alkaloids, xanthones, secoiridoids, terpenes, and phthalides (Ngbolua *et al.,* 2014c). Important phytochemicals such as saponins, flavonoids, terpenoids, alkaloids, and steroids are present in the leaf, stem–bark and root bark of *A. grandiflora* (Odeghe *et al.,* 2012a). Reducing sugar, tannin, phlobatannins, glycosides were found to be absent in both the leaf and the stem bark of *Anthocleista grandiflora* (Sharaibi *et al.,* 2014), however, tannins are present in the root bark and anthraquinones in the leaves. It has been reported that complex indole alkaloids appear to be absent in *Anthocleista grandiflora*, while secoiridoids and alkaloids derived from these during the isolation procedure are present (Mulholland *et al.,* 2005). The secoiridoids found in *Anthocleista grandiflora* are typical of those discovered in two Gentianaceae species, swertiamarin (2) and sweroside (1). Secoiridoid glycosides are in charge of the species' bitter flavour. It is a collection of the chemical compounds extracted from *Anthocleista grandiflora*, together with information about their structures and classes of phytochemicals, such as xanthones, phytosterols, triterpenes, alkaloids, and others. In the leaves and roots of *Anthocleista grandiflora*, tetraoxygenated xanthones and the secoiridiod glycosides sweroside (1) and vogeloside have been found. Sweroside has revealed significant anti-inflammatory activity (Baba and Usifoh, 2011; Babalola *et al.,* 2012). Tene *et al.* (2008) was the first to report the isolation and structural elucidation of a

rearranged nor-secoiridoid, anthocleistenolide from the stem bark of *Anthocleista grandiflora*. Two iridoid glucosides, grandifloroside and methyl grandifloroside, one coumarin, scopoletin (27) and the secoiridoid sweroside (1) were found in *A. grandiflora* (Ateufack *et al.,* 2014).). Some compounds which have been isolated from the stem bark of *A. grandiflora* are grandiflorol, bauerenol (15), bauerenone, 6-ketobauerenone; scopoletin (28) and (þ)-de- methyllasiodiplodin (29); while lupenone (16) and the iridoid sweroside (1) were additional compounds isolated from the root bark (Mulholland *et al.,* 2005).

2.4 Pharmacological activities of *Anthocleista grandiflora*

Antidiabetic activity

In African traditional medicine, the leaves, stems and roots of *Anthocleista grandiflora* are prepared as a decoction or macerated in water or alcohol, and the solution is given orally as a treatment for diabetes in Guinea, Nigeria, Togo, Ghana and Cameroun (Olowokudejo *et al.,* 2008; Jiofack *et al.,* 2010; Diallo *et al.,* 2012). Additionally, more active substances from *Anthocleista grandiflora* should be extracted in order to assess their antidiabetic effect. There has been no research done on the mechanisms of action of the extracts/fractions of these plants.

Antiplasmodial activity

A significant health issue in Africa is malaria, which is brought on by Plasmodium species and spread by mosquitoes. *Anthocleista grandiflora* is used to treat malaria in countries like Nigeria, Mali, Côte-d'Ivoire, Guinea, South Africa and Tanzania, of which the mode of preparation is decoction/maceration and administered orally (Atindehou *et al.,* 2004). Research has involved both in vitro and in vivo antiplasmodial studies on *Anthocleista grandiflora*. Interestingly, the in vivo studies have yielded positive results, indicating efficacy against malaria parasites.

Nevertheless, it's important to note that the in vitro results for *Anthocleista grandiflora* have either been negative or have not yielded particularly impressive findings. In two different in vitro studies by Bapela *et al.* (2014) and Asase and Oppong-Mensah, (2009). Similarly, Bapela *et al.* (2014) reported that 10 mg/mL of dichloromethane and methanol extracts of *A. grandiflora* showed no in vitro antiplasmodial activity (IC_{50} 50 µg/mL). Again, among the 31 plants tested for in vitro antiplasmodial activity by Nondo *et al.* (2015), ethanol extracts of *A. grandiflora* at 100 µg/mL was among the least active with growth inhibition rate of less than 30% against chloroquine-resistant Plasmodium falciparum Dd2 strains. Since most of the in vitro antiplasmodial investigations on the species were not dosage dependent and used a variety of extraction solvents from nonpolar to polar, as well as insufficient laboratory procedures, they appear to be unsatisfactory. Ikegbunam *et al.* (2014) reported the effectiveness of the methanol extract of *A. grandiflora* in increasing the haematological values (PCV, Hb, WBC, platelets, lymphocyte, neutrophils and monocyte) and decreasing the levels of AST and ALT activities of malaria parasite infected rodents. This indicated that the extract possess the ability to enhance blood component to phagocytose, delay or prevent the incidence of anaemia, protect the liver by its free radical scavenging activities and improve the disease progression (Gbadamosi, 2014). As is typical with herbal medicines worldwide, the practice often involves combining multiple plants to address specific disease conditions. This approach is based on the belief that the active compounds in these various plants work together synergistically to produce the desired therapeutic effects. To date, only one isolated compound from *Anthocleista grandiflora*, known as decussatin, has been examined for its antiplasmodial activity, as reported by Ayoka *et al*. (2014). However, the outcomes of in vivo studies lend support to the traditional utilization of *Anthocleista grandiflora* in the treatment of

malaria. In order to find the most suitable or appropriate extraction solvents or standard methodologies for the in vitro antiplasmodial investigations, further research is necessary to understand the differences between in vitro and in vivo antiplasmodial results (Igoli *et al.,* 2005). False negatives are the term used to describe the occurrence of getting positive in vivo results while seeing weak or negative in vitro antiplasmodial results. Similar circumstances have been recorded with nucleoside antiviral medicines, according to Awah *et al*. (2010). In many situations, the results of the in vitro testing may not fully reflect the substances' genuine therapeutic potential because these tests often yield more favourable results when conducted on living organisms. This emphasises the significance of performing in vivo investigations to better comprehend the usefulness of such drugs. In addition, it is important to identify the bioactive elements in the species that are responsible for the antiplasmodial activity in order to understand their mode of action and look into the species for potential novel malaria medications (Diallo *et al.,* 2012).

Antimicrobial activity

Anthocleista grandiflora has shown useful in treating a variety of microbial infection-related illnesses, including bronchitis, candidiasis, typhoid, mycosis, and fever. Additionally, *Anthocleista grandiflora* is a widely used herbal remedy for the treatment of sexually transmitted illnesses in nations including Nigeria, Togo, Cameroon, Gabon, and Equatorial Guinea. (Atindehou *et al.,* 2002; Chah *et al.,* 2006). In addition, Anthocleista grandiflora is used to treat skin conditions such rashes and eczema (Olowokudejo *et al.,* 2008). Usually, the plants are macerated or soaked in water before being consumed. *Anthocleista grandiflora* has exhibited antibacterial activity against pathogenic microorganisms such as *Staphylococcus aureus,*

Escherichia coli, Klebsiella pneumonia, Salmonella typhi, and *Corynebacterium diphtheriae*. According to Baldé *et al.* (2015), *E. coli* is the primary cause of food poisoning and urinary tract infections. Impetigo, cellulitis, boils, abscesses, wound infections, pneumonia, food poisoning, and toxic shock syndrome are only a few of the illnesses caused by *S. aureus*. Typhoid fever is caused by the main pathogen *Salmonella typhi* and is characterized by symptoms like weakness, headache, abdominal pain, and constipation, with rare instances of vomiting and diarrhea on occasion.. It is crucial to remember that, if left untreated, typhoid fever can be a dangerous and even fatal condition. According to Olorunnisola *et al.* (2015), C. diphtheriae causes diphtheria while *K. pneumoniae* causes pneumonia and infections of the urinary tract. These findings assist to explain why local communities and traditional healers use *Anthocleista grandiflora* to treat ailments like skin illnesses, typhoid, diarrhea, and other infections. The long-standing conventional use of these plants in the treatment of microbial infections has been supported by empirical evidence provided by scientific investigations, demonstrating their efficacy in certain therapeutic uses. Although, the traditional use of *Anthocleista grandiflora* for the treatment of STDs and skin diseases still remains to be proven scientifically, their use in the treatment of bacterial and fungal diseases have been sufficiently supported by the above scientific studies (Akinyemi and Ogundare, 2012). Nonetheless, additional research is of utmost importance to pinpoint and isolate the bioactive compounds present in the various extracts of *Anthocleista grandiflora* for use in antibacterial and antifungal applications. It's worth noting that there is currently no reported research on the antiviral activities of *Anthocleista grandiflora*, indicating an area where future investigations could be valuable.

Analgesics, wound healing, Anti-ulcerogenic and anti-inflammatory activities

These findings substantiate the traditional use of *Anthocleista grandiflora* for its potential as a pain reliever inflammatory conditions and in the treatment of wounds. The scientific evidence supporting the traditional use of *A. grandiflora* for treatment of chest pains and wounds respectively has been established, based on evidence of *Anthocleista grandiflora* as anti-inflammatory agents, there is need to research their mode of action for reducing pain and/or inflammation, likewise the bioactive agents mediating these effects (Chah *et al.,* 2006).

Antioxidant activity

Antioxidants play a critical role in preventing the generation and reducing the levels of reactive oxygen and nitrogen species. These reactive species are produced within the body and can cause damage to various biomolecules, including DNA, proteins, and lipids. Antioxidants are widely used in dietary supplements and have been investigated for the prevention of diseases such as cancer, coronary heart disease and even altitude sickness (Baillie *et al.,* 2009). Plants naturally produce a diverse range of antioxidants for their own defense, and some of these compounds, including vitamin C, vitamin E, flavonoids and carotenoids, can also offer health benefits to humans. *Anthocleista grandiflora* is considered a potential source of antioxidants, which could contribute to its health-promoting properties. These antioxidants may help combat oxidative stress and its associated damage to cellular components, offering potential health benefits to those who consume or use this plant. Nonetheless, the compounds within the plants that are responsible for its antioxidant properties are yet to be identified (Bansal and Bilaspuri, 2011).

Fertility activity

Anthocleista grandiflora serves as a source of traditional remedies for addressing issues related to male and female fertility in several African countries, including Nigeria, Togo, Congo, Cameroon, Ghana, Gabon, and Equatorial Guinea. In contrast to the use of *Anthocleista grandiflora* to enhance fertility, the roots is reported to be used as contraceptives or to induce abortion (Kadiri, 2009; Diame, 2010). Reactive oxygen species are important mediators of sperm dysfunction (Wang et al., 1997; Bansal and Bilaspuri, 2011). MDA, a byproduct resulting from the oxidation of lipids, has been documented in sperm cells. Muanya and Odukoya (2008) studied the effect of 9 medicinal plants on lipid peroxidation as an index of male fertility. Lipid peroxidation in raw and cooked fish homogenates was measured as the amount of thiobabituric acid reactive sample (TBARS) in nmol/mg. (Oladimeji Igbalaye, Coleshowers, 2014). In the female reproductive system, estrogen plays a pivotal role, especially in the process of ovulation.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Apparatus and Equipment Used

The apparatus and equipment used in this study include; conical flask, beaker, mortar and pestle, filter paper, measuring cylinder, funnel, crucible, spatula, cages, dissecting set and intubation tubes, micropippete, Analytical weighing balance (Ohaus Corp. Pine Brook, NJ USA., China), spectrophotometer (721G,Zhejiang Top Cloud-Agri Technology Co., Ltd., China), water bath (Equitron Mumbai India, 400013), oven (STH 585 Dionex), centrifuge (90(1) Alpin Medical, England), refrigerator (UK).

3.1.1 Chemicals and Reagents

The following chemicals and reagents were employed, including methanol (JDH), chloroform, normal saline, tween-80, distilled water, and various kits such as ALT, AST, ALP, albumin, and total protein kits. These kits and reagents were procured from Randox Laboratories Limited, situated at Country Atrium in the United Kingdom.

Collection and Authentication of Plant Materials

For this study, the leaves of *Anthocleista grandiflora* were utilized. Fresh leaves of *Anthocleista grandiflora* were collected from the bush in Igbanke village, situated in Benin City, Edo State, Nigeria. These leaves were authenticated and verified by Dr. H. A. Akinnibosun from the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City. To serve as a reference for future use, a voucher specimen with the reference number UBH-W44 was deposited in the herbarium of the department.

3.1.3 Preparation of Extract

The fresh leaves of *Anthocleista grandiflora* were washed with clean water and left to air-dry until they reached a consistent weight over a period of three weeks. To completely remove moisture, the leaves were further oven-dried at 40^{o}C for 4 hours. The dried material was then finely powdered using a mechanical grinder and stored in a clean, airtight amber bottle. A total of 1300 g of the powdered material was subjected to cold maceration with 3 liters of methanol for a duration of 72 hours. After this process, the resulting solution was filtered using wire gauze and a sieve with pores. The filtrate was collected and subsequently concentrated to dryness using a water bath at 40°C. The concentrated extract was preserved in a clean glass container in a refrigerator until it was ready for use. The weight of the concentrate was taken, and the percentage yield calculated.

3.1.4 Experimental Animals

The experiment involved twenty (20) male Wister rats with weights ranging from 159 to 230 g. The rats were purchased from the Laboratory Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria and kept at the same Animal House of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria, where they were used for the experiment. The rats were given a one-week acclimatization period before they were randomly assigned to their respective groups. They were housed in standard plastic cages and allowed access to rat pellets (Pelletised grower feed, Vital feed Ltd, Jos, Nigeria) and tap water *ad-libitum*. All experimental animals were treated in accordance with both institutional and international guidelines governing the use of experimental animals (Pub No. 85-23, revised 1985; Ozolua *et al*., 2009).

3.2 ACUTE TOXICOLOGICAL ASSESSMENT

The oral median lethal dose (LD_{50}) of the methanolic leaf extract of *Anthocleista grandiflora* was evaluated by using modified Lorke method (Lorke, 1983). In the initial phase, nine mice were randomly divided into three groups, with each group consisting of three mice (both sexes represented). These groups were subjected to different doses of the extract, specifically 10 mg/kg, 100 mg/kg, and 1000 mg/kg of body weight, respectively. The mice were closely monitored for a period of 24 hours. As no fatalities occurred during this first phase, the study proceeded to the second phase. In the second phase, a separate set of rats was used, and doses of 2000 mg/kg, 3000 mg/kg, 4000 mg/kg, and 5000 mg/kg of the extract were administered to individual rats. After the administration of the extract, animals were observed for death and symptoms of toxicity within three days in the first instance and then for 30 min each day for another eleven days (Ozolua *et al*., 2010). Gross toxicological symptoms were monitored and the LD_{50} was calculated as follows;

$LD_{50} = (D_0 + D_{100})$.

Where: D_0 = Highest dose that gave no mortality

D_{100} = Lowest dose that produced mortality.

3.4 EXPERIMENTAL DESIGN

Following the results of the acute toxicity test, which showed no fatalities at a dose of 5000 mg/kg, three doses of the extract (200 mg/kg, 400 mg/kg, and 800 mg/kg) were selected for further testing. The selection of these doses was based on the method employed by Bautista *et al*. (2004), Ozolua and Uwaya (2013), and Prasanth *et al*. (2015). Twenty male rats were randomly divided into four groups, with each group containing five rats. Group I served as the control, and

these rats were given normal rat feed along with distilled water, which served as the vehicle for the extract. Rats in Groups II, III, and IV were administered doses of 200 mg/kg, 400 mg/kg, and 800 mg/kg of *Anthocleista grandiflora* leaf extract, respectively. Both the vehicle and the extract were orally administered once daily using an orogastric tube for a duration of 28 days. At the conclusion of the experiment, the final body weights and the results of biochemical assays were determined.

3.5 SAMPLE COLLECTION

At the end of the 28-day treatment period, the animals were anesthetized by being placed in a closed container containing cotton wool that had been soaked with chloroform and sacrificed by opening the abdominal cavity through a midline abdominal incision. Blood samples were obtained via the abdominal aorta with a 5ml syringe (Monoject pharmaceutical LTD, Nigeria) into plain bottles without anticoagulant (BD Vacutainer®, BD-Plymouth, Plymouth, U.K) (Ozolua *et al*., 2009). The blood samples were allowed to clot and the serum was obtained by centrifuging at 3000 revolutions per minute (rpm) for ten minutes using a table top centrifuge (90(1) Alpin Medical, England) (Ozolua *et al*., 2010). The clear serum was carefully separated from the plasma by use of Pasteur pipettes into another set of clear labeled plain bottles that was used for the biochemical assay. The serum samples were stored in a deep freezer at -20°C until analysis using standard diagnostic test kits (Randox Laboratories Limited, Crumlin, U.K.) on an automated spectrophotometer.

EVALUATION OF BIOCHEMICAL PARAMETERS

Determination of Urea Concentration: Urea concentration was determined using Urease Berthelot method as described by (Fawcett, 1960; Anuforo *et al*., 2020) based on the principle that urea in serum is hydrolysed to ammonia in the presence of urease and the ammonia measured spectrophotometrically at wavelength of Hg 570nm on reacting with hypochlorite and phenol (Berthelot reaction) to form a blue coloured indophenol compound. The concentration of Urea was calculated using the following formula;

Urea Conc. (u/l) = $\frac{Abs.of\ sample}{Abs.of\ standard}$ x Concentration of standard (mg/dl).

Determination of Creatinine Concentration: Creatinine concentration was determined using direct endpoint method as described by (Henry, 1974; Anuforo *et al*., 2020). This method relies on the reaction of creatinine with picric acid under alkaline conditions, resulting in the formation of a colored complex that can be detected at 510 nm. The absorbance of each test tube was measured and recorded, and the concentration of creatinine was calculated as follows;

Creatinine Conc. (u/l) = $\frac{Abs.of\ sample}{Abs.of\ standard}$ x Concentration of standard (mg/dl).

Determination of Chloride Concentration: Serum chloride ion concentration was determined based on the colorimetric estimation of red colored complex formation from the reaction of the sample (or the standard chloride) and chloride reagent mixed and incubated at 25 ^{o}C for 5 minutess, and read at 500 nm (Anuforo *et al*., 2020). The absorbance of each test tubes was read and recorded and the concentration of Chloride was calculated as follows;

Chloride Conc. (u/l) = $\frac{Abs.of\ sample}{Abs.of\ standard}$ x Concentration of standard (mg/dl).

Determination of Sodium Concentration: The method employed in this study is based on the precipitation of sodium as a triple salt, as specified by the kit manufacturer, Hi-Tech Diagnosis Nigeria Limited. In this chemical reaction, any exccess uranium present after the precipitation of the triple salt (known as Sodium magnesium uranyl acetate) reacts with ferrocyanide. This reaction generates a chromophore, the absorbance of which changes in an inverse relationship with the concentration of sodium in the test specimen. The absorbance of each test tubes was read and recorded and the concentration of Sodium was calculated as follows;

Sodium Conc. (u/l) = $\frac{Abs.of\ sample}{Abs.of\ standard}$ x Concentration of standard (mg/dl)

Determination of Potassium Concentration: Potassium ion concentration was determined using the turbidometric method as described by (Henry, 1974; Anuforo *et al*., 2020) based on the principle that the extent of turbidity is proportional to the potassium concentration as measured spectrophotometrically at 578 nm. The absorbance of each test tubes was read and recorded and the concentration of Potassium was calculated as follows;

Potassium Conc. (u/l) = $\frac{Abs.of\ sample}{Abs.of\ standard}$ x Concentration of standard (mg/dl).

BODY WEIGHT MEASUREMENT

The body weight of all experimental animals was measured using a digital electronic balance before the initial oral administration, and these measurements were then taken on a weekly basis until the final day of the oral administration of the extract.

STATISTICAL ANALYSIS

Results were expressed as mean ± standard error of mean (SEM) and n represents the number of animals per group from which blood and semen samples were obtained. Data comparisons

between treated and control groups were made using one- way analysis of variance (ANOVA) with Tukey post hoc test (SPSS version 20). $P < 0.05$ indicates statistically significant difference.

CHAPTER FOUR

RESULT

Table 4.1: Mean values of kidney function test on male rats

Parameters	Control Group	Group 1 (200mg/kg)	Group 2 (400mg/kg)	Group 3 (800mg/kg)
Urea (mg/l)	42.20 ± 3.01	39.40 ± 1.36	52.60 ± 3.91	51.20 ± 2.62
Sodium (mmol/l)	139.80 ± 0.73	144.60 ± 4.03	144.00 ± 2.03	144.4 ± 2.50
Potassium (mmol/l)	6.84 ± 0.50	14.94 ± 1.12****	13.74 ± 1.28***	11.76 ± 0.41**
Bicarbonate (mmol/l)	19.80 ± 0.37	18.80 ± 0.86	19.40 ± 0.75	16.60 ± 1.63
Chloride (mmol/l)	107.80 ± 0.37	112.20 ± 3.99	113.40 ± 4.55	104.80 ± 2.63
Createnine (mg/dl)	0.84 ± 0.02	0.68 ± 0.08	0.82 ± 0.09	0.84 ± 0.09

Values are expressed as mean ± SEM (n = 5); statistically, all the parameters measured were not significant when compared with the control using one way analysis of variance (ANOVA) multiple comparisons.

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.0 DISCUSSION

The kidney's functional integrity is to maintain total body homeostasis through its role in the excretion of metabolic wastes and in regulation of intracellular fluid volume, electrolyte composition, and acid-base balance (Orisakwe *et al.,* 2004). This therefore implies that any harmful effect on body metabolism could be suggestive of toxic insult to the kidney (Abubakar *et al.,* 2010). The kidneys play a crucial role in maintaining the optimal chemical composition of body fluids. They achieve this by acidifying urine and eliminating metabolic waste products like urea, uric acid, creatinine, and various ions from the body (Shagun *et al*., 2015).

The primary objective of the current study was to assess the potential toxic impact of *H. umbellata* on renal function. This assessment was carried out by examining the levels of creatinine, blood urea, ions, and by observing any histopathological changes in the kidneys. Urea, a byproduct resulting from the breakdown of proteins, constitutes about 90% of the urea produced in the body, and it is primarily excreted through the kidneys (Walmsley *et al.,* 2010). On the other hand, creatinine is a waste product produced from the breakdown of muscle creatinine, which is utilized during muscle contractions. Creatinine is commonly measured as an index of glomerular function (Treasure, 2003). The normal range of serum creatinine is 0.2–0.8 mg/dl for rats (Weber et al., 2002). Since creatinine is solely excreted through the kidneys, any impairment of kidney function can lead to inefficiency in excreting both urea and creatinine, resulting in their accumulation in the bloodstream. Consequently, elevated levels of blood urea and creatinine are indicative of kidney damage or dysfunction.

The findings from the current study indicate that there were no statistically significant differences observed in the combined means of all the parameters measured among the groups. This lack of significance was observed when comparing the groups ($P>0.05$) receiving doses of 500 mg/kg, 1000 mg/kg, and 2500 mg/kg with the control group. There was a significant ($P > 0.05$) increase in urea level in the rats treated with *H. umbellata.* The increase was more at 1000 mg/kg (52.60 ± 3.91) when compared with control (42.20 ± 3.01).

The results of this study indicate that the oral administration of *H. umbellata* methanol seed extract at three different doses to the animals over a 56-day period led to a notable decrease in serum total cholesterol and triglyceride levels.

5.1 CONCLUSION

The nephrotoxic potential of methanol leaf extract of *A. grandiflora* was tested in rats after oral administration for 28 days. The results obtained in this investigation suggest that sub-chronic consumption of methanol leaf extract of *A. grandiflora* at varyinng dosages over a three-week timeframe, has no evidence of kidney toxicity, as indicated by normal levels of urea and creatinine in the blood, as well as the absence of any abnormalities in the kidney tissue examined through histology for all treatment groups.

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