PHYTOCHEMICAL AND TOXICOLOGICAL EVALUATION OF *CROTON MENYHARTHII* FROM TANA RIVER COUNTY, KENYA

EMMA WANJIKU KAGUNYA (BPHARM UoN)

A thesis submitted in partial fulfillment of the requirements for the Degree of Master of Science in Pharmacology and Toxicology of the University of Nairobi

Department of Public Health, Pharmacology and Toxicology

DECLARATION

I hereby declare that this thesis is my original work and has not been submitted to any other University for an academic award.

Emma Wanjiku Kagunya

J56/7684/2017

Signature: _____ Date: 9th November, 2020

This thesis has been submitted for examination with our approval as University supervisors

Professor James Mbaria (BVM, MSc, PhD)

Department of Public Health, Pharmacology and Toxicology,

University of Nairobi.

Signature: _____ Date: 9/11/2020

Dr. Catherine Kaingu (BVM, MSc, PhD)

Department of Veterinary Anatomy and Physiology,

University of Nairobi.

Signature: _____ Date: 9/11/2020

UNIVERSITY OF NAIROBI

DECLARATION OF ORIGINALITY

Name of Student: EMMA WANJIKU KAGUNYA

Registration Number: J56/7684/2017

College: OF AGRICULTURE AND VETERINARY SCIENCES

Faculty/School/Institute: VETERINARY MEDICINE

Department: PUBLIC HEALTH, PHARMACOLOGY AND TOXICOLOGY,

Course Name: MASTER OF SCIENCE IN PHARMACOLOGY AND TOXICOLOGY

Title of the work: PHYTOCHEMICAL AND TOXICOLOGICAL EVALUATION OF *CROTON MENYHARTHII* FROM TANA RIVER COUNTY-KENYA

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DEDICATION

This thesis is dedicated to my lovely children Elvis and Eleanor, who have been my source of inspiration throughout the study period.

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TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ABBREVIATIONS AND ACRONYMS

ALT - Alanine aminotransferase

AST - Aspartate aminotransferase

BUN - Blood urea and nitrogen

Bwt - Body weight

CIDP - County Integrated Development Plan

DCM - Dichloromethane

EDTA - Ethylenediaminetetraacetic acid

GHS - Globally Harmonized System of Classification and Labelling of Chemicals

H&E - Hematoxylin and Eosin

KDHS - Kenya Demographic and Health Survey

LD50 - Median lethal dose

MCH - Mean corpuscular haemoglobin

MCH - Mean corpuscular haemoglobin concentration

MCV - Mean corpuscular volume

NCAPD - National Coordinating Agency for Population & Development

OECD - Organization for economic co-operation development

PHC - Primary Healthcare

PPB - Pharmacy and Poisons Board

RBC - Red blood cells

T&C - Traditional and complementary medicine

TMPs - Traditional medicine practitioners

TRM - Traditional Medicine

UN - United Nations

WBC - White blood cells

WHO - World Health Organization

ABSTRACT

The use of plants for primary health care has increased tremendously over the last few decades especially in developing countries. However, the health benefits claims and safety profile of most plants is yet to be validated. *Croton menyharthii* is an indigenous plant in Kenya and has been used to treat reproductive health ailments and for fertility regulation in Tana River County. Despite its use, its toxicological and phytochemical profile is yet to be validated. The main objective of this study was to determine the phytochemical composition of *Croton menyharthii* root bark extracts and to evaluate its acute and sub-acute toxicity.

Air dried root barks of the plant were obtained from Tana River County and were ground to powder. The powder was used to make aqueous and dichloromethane (DCM)/Methanolic extracts. Qualitative phytochemical analysis was done on both extracts. The acute oral toxicity study protocol was conducted according to the Organization for the Economic Co-operation and Development (OECD) test guideline 423 using twenty-four mature female wistar rats. The sub-acute oral toxicity protocol was carried out as per the OECD test guideline 407 using 40 mature female wistar rats. Three graded doses of 200, 400 and 800 mg/kg body weight of both aqueous and DCM/Methanolic extract of *Croton menyharthii* root bark were administered.

Both aqueous and DCM/Methanolic extracts revealed presence of alkaloids, saponins, tannins, phenols and cardiac glycosides. The LD_{50} for both the aqueous and DCM/Methanolic extracts was found to be above 2000 mg/kg body weight. In both acute oral and sub-acute toxicity studies, there were no significant clinical symptoms observed apart from lethargy and sedation that resolved within four hours. Rats that received a single dose of 5000 mg/kg body weight *C. menyharthii* aqueous extract had a significant ($P<0.013$) low body weight gain compared to the control. There were however signs of irritation among the rats that received the DCM/Methanolic extract. All rats showed remarkable gross necropsy findings.

In the sub-acute toxicity study, there was a significant ($P<0.016$) increase in feed consumption among the rats that received 400 mg/kg body weight aqueous extract and 200 mg/kg body weight DCM/Methanolic extract ($P<0.03$) compared to their respective controls. There was also a significant ($P<0.04$, $P<0.003$) more water consumption in the rats that received 200 and 400 mg/kg body weight *Croton menyharthii* aqueous extract respectively compared to the control. Average water and food consumption reduced over time in rats that received the DCM/Methanolic extract. There was a non-significant increase in mean body weight of all treatment rats compared to their respective controls.

Both extracts had a non-significant effect on haematological parameters. However, there was a significant ($P<0.006$, $P<0.00$, $P<0.00$) dose related decrease in serum protein levels in the treatment groups that received the *C. menyharthii* aqueous extract. There was a significant ($P<0.045$) lower relative-organ weight ratio of the liver from the rats that received 200 mg/kg body weight dose of the DCM/Methanolic extract. For both extracts, histopathology of all treatment groups revealed diffuse portal blood vessel congestion in the liver and parenchymal blood vessel congestion in the kidneys. In addition, treatment groups that received DCM/Methanolic extract revealed dose related lymphocytic infiltration and multifocal hemorrhage of renal parenchyma indicating renal tubular injury.

In conclusion, *C. menyharthii* root bark extracts are of pharmaceutical importance since they contain useful phytochemicals that can be explored for drug discovery. They can be deemed safe when used as single doses of < 2000 mg/kg body weight. However, on long term use, they can be nephrotoxic. Quantitative phytochemical analysis of *Croton menyharthii* needs to be done. Kidney function needs to be monitored in long term use due to the possibility of nephrotoxicity. A detailed study needs to be done to reveal the effects of the extract on body weight gain, food and water consumption.

CHAPTER ONE

INTRODUCTION

1.1 Background information

The use of plants as medicine and supplements is widely practiced traditionally in most parts of the world. Plants use has increased immensely over time with more than 80% of people globally relying on them for primary health for both minor and chronic illnesses (Barnes, 2003; Ekor, 2014). Plants therefore play a major role in Traditional Medicine (TRM) and has great prospects in healthcare delivery globally especially in countries that are developing (Kayombo *et al.*, 2013) . Over the years, a plethora of mastery on use of herbal medicine has accrued and the knowledge is easily accessible for contemporary scientific research on drug discovery (Pan *et al.*, 2013).

The possibility of success in developing a new drug from medicinal plants as opposed to chemical synthesis is higher due to the lengthy history of herbal medicine use in curbing various ailments in indigenous societies (Pan *et al.*, 2013). Approximately 25% of conventional drugs are derived from their traditional medicinal use. There is hope of discovering novel drugs from medicinal plants for resistant diseases and others whose cure is yet to be discovered (Kayombo *et al.*, 2013).

The use of plants has shown promising potential and the efficacy of most of them has been established through clinical trials and systematic reviews. Nevertheless, majority of these plants are yet to be toxicologically analyzed and their use is poorly regulated (Barnes, 2003; Ekor, 2014). There is therefore minimal understanding of their mode of action, food interactions, possible side effects, contraindications and drug interactions. Hence, safety evaluation of these plants remains a key issue (Barnes, 2003; Ekor, 2014).

Among the many traditional uses of plants is the use of herbal medicine to control and regulate fertility in humans. Due to ease of access, affordability and perceived less side effects of

traditional medicine, most women, especially those who abide in rural areas of developing countries and have inadequate access to modern contraceptives, use herbal plants to manage reproductive issues (Anand *et al.,* 2015; Tsobou *et al.,* 2016). However, despite the fact that most herbal contraceptives used are believed to be safe and effective, minimal data is available on the same. The effects may also vary with the seasons and the biogeographical regions (Anand *et al.,* 2015).

A study in Tana River County identified and documented plants that are used by traditional medicine practitioners (TMPs) for the management of female reproductive health challenges. Among the plants, is *Croton menyharthii,* and it had the highest significance (Kaingu *et al.,* 2013). The plant is used to treat infertility, prolonged irregular menses, post-partum hemorrhage, threatened abortion and for fertility regulation. The root barks or the leaves are boiled and half a glass of the decoction taken orally three times a day for five days (Kaingu *et al.,* 2013).

A subsequent study was done to validate the claims and it was indeed found that, *Croton menyharthii* had anti-fertility and anti-implantation properties (Kaingu *et al.,* 2017). It caused a significant dose-dependent rise of progesterone and a degeneration of corpora lutea in adult female wistar rats (Kaingu *et al.,* 2017). Despite the use of the plant as a fertility regulator, its toxicity profile is yet to be determined. The phytochemicals compounds are still unknown. Kaingu *et al., 2017* recommended evaluation of the toxicological and phytochemical evaluation of *Croton menyharthii* as a step in the validation process towards generation of a novel contraceptive drug.

1.2 Statement of the problem

In an attempt to generate a novel contraceptive, one that is safe, efficacious, convenient, affordable and reversible, research has turned to explore herbal medicines (Bala *et al.*, 2014). *Croton menyharthii* has widely been used in Tana River County-Kenya as a fertility regulator and its effects have been proven. It therefore can be explored for discovery of a novel fertility control agent. However, its toxicity and phytochemical profile remains unknown (Kaingu *et al.*, 2017).

1.3 Objectives of the study

1.3.1 General objective

To evaluate the phytochemical and toxicological profile of *Croton menyharthii* root bark extract.

1.3.2 Specific objectives

1. To determine the preliminary phytochemical compounds of *Croton menyharthii* root bark extract.
2. To determine the acute oral toxicity of *Croton menyharthii* root bark extract.
3. To determine the sub-acute toxicity of *Croton menyharthii* root bark extract.

1.4 Null hypothesis

Croton menyharthii root bark extracts are toxic and do not have any bioactive phytochemical components.

1.5 Justification

There is a global increasing demand for herbal medicine. Medicinal plants are affordable and easily accessible for most people (Zhang, 2015). However, there are concerns regarding their use and safety since among the herbal products in the global market, not more than 10% are actually standardized (Ifeoma and Oluwakanyinsola, 2013).

In many states, herbal medicine does not undergo as rigorous regulatory control as the conventional medicine in terms of efficacy and safety hence the uncertainty in their use. Toxicity testing aims to reveal the presence or absence of any risk that may develop with use of medicinal plants, therefore avoiding possible adverse reactions (Subramanian *et al.*, 2018). *Croton menyharthii* was among the highly mentioned fertility regulators in Tana River County(Kaingu *et al.*, 2013). A subsequent study proved that it indeed has effects on the reproductive system of female wistar rats (Kaingu *et al.*, 2017). Despite its wide use, its toxicological and phytochemical profile has not been established (Schmelzer, 2010).

CHAPTER TWO

LITERATURE REVIEW

2.1 Traditional medicine and complementary medicine

Traditional medicine (TRM) is the use of expertise and practices based on the ideologies, convictions and customs native to different societies, in the maintenance of health (WHO, 2004). It is a vague approach that consists of a range of well-established and still emerging practices based on various beliefs and theorems (Xue, 2008). The terms alternative, complementary, or non-conventional medicine are sometimes used in place of traditional medicine (WHO, 2002). TRM is one of the primary sources of health care due to the escalating levels of chronic diseases, antimicrobial resistance and increasing health care costs (Zhang, 2015). There are several types of traditional medicine and each is unique to the predominant conditions, ecosystem, and the area within which it first developed (WHO, 2005).

Ayurvedic medicine which is a natural system of treatment has been in existence for more than 5,000 years in India. Similarly, in China, Traditional Chinese Medicine originated roughly 3,000 years ago and is in practice all over East Asia. Other forms of traditional medicine are: Traditional Korean Medicine, Traditional Unani Medicine and Traditional Native American Medicine, which are from different parts of world and have been in existence for long. In all these systems, herbs and herbal products are used (Prasad and Tyagi, 2015) among others such as mineral derived medicines, psychological therapies, work outs and animal products (Peltzer, 2009). There has been an ample rise in the use of Traditional medicine and complementary/alternative medicine (T&CM) in recent years (WHO, 2004).

2.2 Phytotherapy

This is the use of plants in treating, preventing and managing health problems (Ameh *et al.*, 2010). Medicinal plants have therapeutic properties and exert a pharmacological effect that is beneficial to the body (Motaleb, 2011). Plants have the potential to be used in drug discovery and development (Rasool, 2012). Indeed, they have been used for many years for the treatment and prevention of health ailments (Singh, 2015).

There is wide distribution of medicinal plants all over the world and across diverse habitats. A number of medicinal plant species (35,000-70,000) with biological effects have been identified. This includes 10,000-11,250 species in China, 2527 in North America, 7500 in India, and 2237 in Mexico (Devi, 2017). Out of these, 800 species are used for commercial manufacture of drugs and roughly 90% of them are obtained from the jungle. Most of the plants are trees. This accounts for 33% of all the species used. The rest are herbs (32%), shrubs (20%), grasses and climbers (12%) and others like ferns, algae and lichens (3%) (Devi, 2017).

2.3 Rationale behind use of medicinal plants

The need to treat illnesses led to the awareness of medicinal plants as potential drugs. Through trial and error, man learnt to acquire drugs from plants parts such as seeds, leaves, barks, fruit and roots (Petrovska, 2012). Majority of people still rely heavily on medicinal plants due to their spiritual belief, social-economic status and limited access to alternative health facilities (Rijal, 2008). In addition, conventional medicines are costly. Therefore, the use of herbal medicines whether in their crude or processed form is acknowledged (Prasad and Tyagi, 2015). Medicinal plants are readily available and hence 80% of people in developing countries use them (WHO, 2004). Others use them to avoid adverse effects of synthetic medicines (Zhang, 2015). In addition, there is a general belief that medicinal plants are non-toxic since they are natural. This is not always true especially when combined with other herbs and or conventional medicines (Canter and Ernst, 2004; Cohen and Ernst, 2010; Loya *et al.*, 2009). Historic and

cultural practices of a country sometimes influence the use of traditional medicine. For example, Republic of Korea and Singapore have 76% and 86% of their respective populations still using TRM yet they both have a well-established conventional health care system. In China, TRM is used as complementary therapy (Zhang, 2015).

2.4 Trends in use of medicinal plants

Plants have played a major role throughout human history in the discovery of new medicines. They occupy a significant part in the treatment of diseases globally (Prasad and Tyagi, 2015). Indigenous knowledge conforms to local circumstances, conceptualized and passed on from generations to generations (Devi, 2017). In the early 19^{th} century, the segregation of active moieties such as morphine, quinine and strychnine from alkaloids, marked a new era of plants use as medicine and was the dawn of intense research of medicinal plants (Saranraj *et al.,* 2016).

With time, emphasis shifted to synthetic drugs but due to their overly high adverse effects, the interest in novel compounds from plants has grown steadily. Identification and isolation of chemically active molecules has enabled use of crude plant extracts to be replaced with purified substances; for example, quinine and aspirin; which are more potent and easily administered (Saxena *et al.,* 2013).

2.5 Modern Phytomedicine

Quite a number of new drugs have been derived from natural products (secondary metabolites) and their derivatives (Li and Vederas, 2009). Despite the numerous challenges hindering drug discovery from herbal medicine, natural products derived from plants still play a significant role in the discovery of new lead compounds (Jachak and Saklani, 2007). Some vital conventional medicines have been developed from novel prototype bioactive molecules obtained from plants (Salim *et al.,* 2008).

The secondary metabolites in plants are capable of having pharmacological effect and this forms a basis of their consideration as a potential source of drug discovery (Shakya, 2016). They can be used in their native forms as medicine or as drug precursors, for example paclitaxel, diogenin and oseltamivir phosphate. They can also be used as templates for synthetic modification for example, guanidine and atropine or as pharmacological probes for example, genistein and phorbol (Salim *et al.,* 2008).

The pursuit for drug invention from plants is ongoing and the likelihood of succeeding is much higher than from chemical synthesis (Pan *et al*., 2013). Many herbal plants used today as conventional medicine have the same or identical therapeutic application as their indigenous use (Fabricant and Farnsworth, 2001).

Through contemporary science, the bioactive components of the plants are determined and their mode of action investigated (Prasad and Tyagi, 2015). Globally, drugs attained from plants account for approximately 25% of current prescriptions (Galor and Benzie, 2011).

Some plants that have led to commonly used conventional medicine are *Salix alba* (acetysalicylic acid), *Papaver somniferum* (morphine and other opiod alkaloids*), Digitalis purpurea* (digitoxin and analogues), *Cinchona succirubra* (quinine), *Taxus brevifolia* (paclitaxel), *Artemisia annua (*Artemisin) and *Pilocarpus jaborandi* (pilocarpine) (Dias *et al.*, 2012).

2.6 Role of WHO in use of traditional medicine

The number of persons using medicinal plants is gradually increasing and this has led World Health Organization (WHO) to come up with guidelines to ensure rational use, efficacy and safety. The organization also provides a guideline for effective future conservation (WHO, 1993) of the plants. As part of its mandate in saving lives and promoting health, WHO supports the incorporation of T&CM into health systems through development of national policies (WHO, 2013). WHO has also developed and provided universal standards, scientific

guidelines and strategies for research into T&CM's products, use and regulation (WHO, 2013). The research is directed towards the safety, efficacy and scientifically proven use of T&CM (WHO, 2004).

2.7 Phytochemicals screening of medicinal plants

Phytochemicals are pharmacologically active chemical compounds naturally found in plants. They are also called secondary metabolites and their main purpose is to protect the plant against potential hazards such as contamination, disease and predators (Oliveira, 2015). They are used in humans for disease prevention and health maintenance. A lot of research has been geared towards understanding their mode of action and to validate their efficacy (Saxena *et al.,* 2013; Saranraj *et al.,* 2016).

The phytochemical compounds are present in a plant as a group of compounds. These Phytochemicals determine the valuable medicinal properties of the plant. This concept is consistent with the fact that the therapeutic properties of plants are distinctive to specific plant species (Barboza *et al*., 2009). Phytochemical compounds are found in the roots, leaves, stems, flowers, fruits, barks and seeds (Altemimi *et al.*, 2017).

Phytochemical evaluation is economically important especially in pharmaceutical companies for the manufacture of novel drugs (Wadood *et al*., 2013). Many studies have been done on the medicinal potential of these compounds (Saxena *et al.,* 2013). Around 4000 phytochemical compounds have been distinguished and classified by their diverse pharmacological actions, physical and chemical properties. They include among others alkaloids, sterols, lignans, phenolic acids, saponins, stilbenes, tannins, terpenoids and flavonoids (Nyamai *et al.*, 2016). Only about 150 of them have been studied in details. Most of them are yet to be identified (Saxena *et al.,* 2013; Nyamai *et al.,* 2016).

2.8 Toxicity testing of medicinal plants

Regardless of the consistent use of medicinal plants, most phytomedicines' safety profile is yet to be validated (Subramanian *et al.,* 2018). Although herbal medicines are considered non-toxic compared to synthetic compounds, some may induce deleterious effects to the users (Mounanga *et al.,* 2015). It is therefore necessary to validate their safety through toxicity studies (Subramanian *et al.,* 2018). Such include acute oral, sub-acute, sub-chronic and chronic toxicity studies (Chanda *et al.,* 2015).

Several toxicity studies have been done on medicinal plants. Some are reported to be safe for example *Momordica dioica* used to manage asthma and leprosy; *Adina cordifolia* used as an antifertility and anti-inflammatory agent (Sharwan *et al*., 2015); *Croton zambesicus (*Okokon *et al.,* 2010), *Spathodea campanulata* (Ilodigwe *et al.,* 2010), *Centella asiatica* (Chivapat *et al.,* 2011), *Asparagus africanus* (Kebede *et al.,* 2016), *Pistacia integerrima* (Sharwan *et al.,* 2015), *Aconium napeilus* Linn, *Artemisia afra* and *Monascus purpureus* (Chanda *et al.,* 2015) all with a large safety margin. Others are reported to be poisonous for example, *Digitalis spp, Atropa belladonna, Aconitum spp, Arnica spp* and pyrrolizidine-alkaloid-containing plants which are hepatotoxic (George, 2011). Other toxic plants include *Acacia karoo, Aframomum melegueta, Tithonia diversifolia, Semecarpus anacrdium, Magnistipula butayei,* and *Euphorbia hirta* (Chanda *et al*., 2015). Hence it is crucial to investigate and document probable adverse effects that may result from the use of herbal medicines before their manufacture and marketing (Subramanian *et al.,* 2018).

2.9 Regulation of herbal medicine

Regulation of herbal medicine in the world has proven to be a difficult task. This is because, various states have diverse definition and categorization of medicinal plants. A single plant can be categorized as a herbal medicine in one country, as food in another, or as food supplement in yet another as per the country's food and drugs regulation. For example, until 2008, *Ginkgo*

biloba L, was categorized as a food supplement in United States of America, as food in United Kingdom and as a medicinal product in Germany (Heinrich, 2015) This has led to confusion among consumers and patients and difficulties in defining the concept of herbal medicine for national drug regulation (Heinrich, 2015; WHO, 2005). The recent rise in interest in use of natural remedies has led to expansion in use of medicinal plants(Chaitanya, 2019). Many people who use herbal products consider them safe as they are from natural source. Health practitioners and policy makers should come up with strategies which will reduce the risks associated with their use (Heinrich, 2015). Most herbal products are complex mixtures whose composition differs with where they are grown, gathered, processed and formulated. Regulation of their use is important in ensuring their safety and efficacy (Zhang, 1998). This includes putting measures across that will ensure the evaluation of their pharmacological, toxicological and chemical properties and therefore insist on evidence based use (Heinrich, 2015). Many countries have put in place systems in a bid to regulate the use of herbal products. For instance, a new regulatory approach for member states of the European Union was developed in 2004 to harmonize the access to and assessment of traditional herbal medicinal products which was mainly based on scientific evaluation and built up evidence over many years (Briggs *et al.,* 2014).

In Kenya, laws and regulations of herbal medicine use and practice are inadequate *(Okumu et al.,* 2017). Kenya is yet to incorporate herbal medicine use in the national drug policy unlike other countries for example, in Asia (Kigen *et al.,* 2013). Kenya Pharmacy and Poisons Board (PPB) is responsible for registration of medicinal herbal products most of which are imports from China and India. Ministry of Gender, Sports, Culture and Social Services has the mandate to register traditional herbalists of which majority comes from urban areas. Those in rural areas are not in the known about registration. PPB should therefore come up with guidelines and

legal framework for registration of herbalists and herbal products in a bid to streamline herbal practice and ensure consumer safety (Ichim, 2019; Kigen *et al.*, 2013).

2.10 *Croton menyharthii*

Croton Menyharthii belongs to the family *Euphorbiaceae*. It is normally known as rough-leaved Croton due to its leaves which have a rough surface of sparse stellate hair. It is a small tree or a much-branched shrub that grows up to two to five meters long. It is distributed worldwide occurring on rocky outcrops, in dry timberlands and in dense marshy thickets, from sea-level up to 1300 meters. In Africa, it's available in Zambia, Angola, Namibia, South Africa, Swaziland, Botswana, Kenya, Malawi, Ethiopia, Tanzania, Somalia, Mozambique, and Zimbabwe (Fen, 2014; Hyde *et al.*, 2018).

Croton menyharthii has been used widely for local medicinal uses. For instance, in Somalia, fresh or dried roots are used to treat dysmenorrhoea while fresh or dried crushed leaves are used to treat tapeworm and hepatitis (Schmelzer *et al.*, 2010). The root bark extract is used for the management of intestinal obstruction. The tree has been used to treat malaria in East Africa. The leaves and twigs have manifested marked anti-plasmodial activity. Others use it to treat influenza (Schmelzer *et al.*, 2010).

In Tana River County-Kenya, *Croton menyharthii* is locally known as "muyama or mualikaji" by the Pokomo people (Kaingu *et al.*, 2013). It has been used to treat prolonged and/or irregular menses, to manage post-partum hemorrhage, infertility, spontaneous abortions and for fertility regulation. The leaves and/or roots are simmered in water and half a glass of the decoction taken daily orally two to three times for five days. Some inhale smoke from its leaves to ease menstrual pains (Kaingu *et al.*, 2013).

The antifertility claims have so far been confirmed as the root bark extracts of the plant significantly disrupted the estrus cycle of female wistar rats and caused a dose dependent significant reduction in fertility and implantation index (Kaingu *et al.*, 2017). Earlier,

investigations of *Croton menyharthii* crude leaf extracts led to identification of three flavonols and an indole alkaloid which showed a marked antimicrobial and enzyme inhibition activities (Aderogba *et al.*, 2013). However, no data is available on the phytochemical composition of *Croton menyharthii* root bark extract nor its toxicity profile (Schmelzer *et al.*, 2010).

Figure 2.1: Aerial view of *Croton menyharthii* plant (Photo by author taken at Ngao village, Tana River County)

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

Croton menyharthii root bark samples were obtained from Tana River County-Kenya, where the plant has been used as a fertility regulator in Garsen, Idsowe and Ngao villages in coastal areas which have a rampant use of herbal medicine (Kaingu *et al.,* 2013). Tana River County is in the coastal area of Kenya between latitude 0^0 and 3^0 South and longitudes $40^0$15' East and $38^0$30'East. Its area is approximately 38,782 km^2 and borders Kitui to the west, Mwingi to the North West, Garissa to the North East, Meru and Isiolo to the North, Ijara to the East, Lamu to the South East, Malindi to the South West and the Indian Ocean (35km coastal strip) to the south (Tana River County Integrated Development Plan, 2013)

According to 2009 census, the county has a population of approximately 240, 075 of which, seventy-two percent of this population live beneath the poverty line. Ninety-six percent of Tana River County lies in the coastal lowland area which experiences low, erratic rainfall. The rainfall is received in two seasons (April-May; October-November) with an annual average of 300-500mm and high temperatures of 30 degrees Celsius. The county is inhabited by diverse ethnic groups; the main ones being Somali, Pokomo, Bajuni, Munyoyaya, Wardei, Giryama, Ormo and Malakoti.

The major economic activity undertaken by most of the residents of Tana River County is agriculture whereby most people practice marginal mixed farming, mixed farming, and pastoralism. Other economic activities include tourism and fishing (Tana River County Integrated Development Plan, 2013).

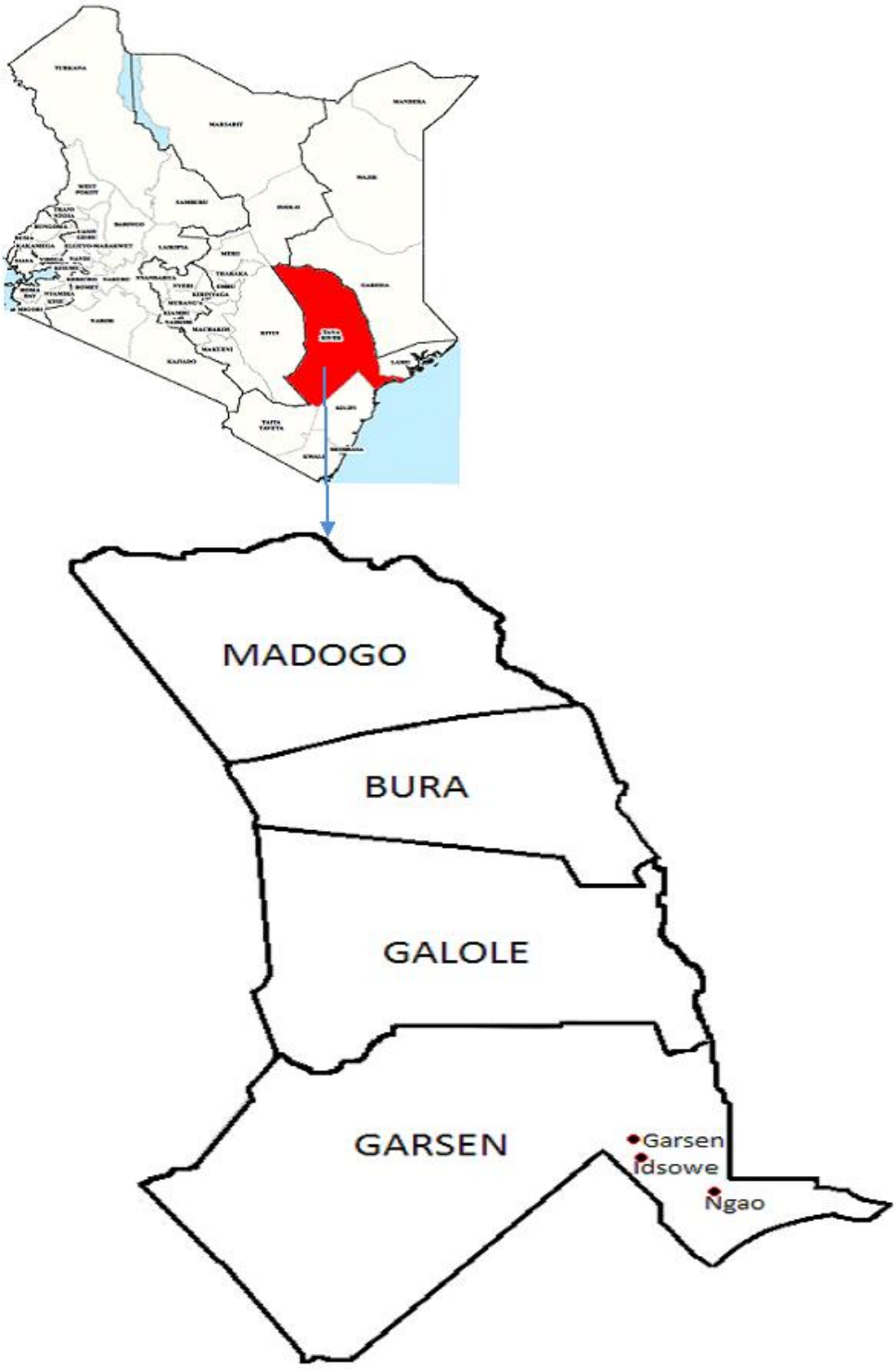

Figure 3.1: Tana River County location in Kenya (**Source**: Tana River County CIDP, 2013: Maphill)

3.2 Sample collection and identification

Fresh samples of *Croton menyharthii* plant were harvested from Tana River County and brought to the School of Biological Sciences at the University of Nairobi where botanical identification was authenticated by a taxonomist. Voucher specimen (CK021) was preserved in the University herbarium for future reference.

3.3 *Croton menyharthii* extract preparation

The plants roots were thoroughly washed with running tap water then dried. The root barks were scrapped off and cut into pieces using a knife. The root barks pieces were spread on a flat surface then air dried at room temperature in a rodent, dust and pest-free, aerated room for two weeks. They were then crushed to a fine powder in a fume chamber using a Cunningham grinder. The plant powder was packaged into 500 grams sachets and stored in a dry, dark, cool and well ventilated cabinet away from direct sunlight.

3.3.1 Aqueous extract

One thousand three hundred grams of *Croton menyharthii* root bark powder was weighed using a Mettler digital weighing balance (Mettler PM4600-DeltaRange®). The powder was immersed in distilled water at a ratio of 1 to 6 (w/v) in a volumetric flask and macerated for 48 hours at room temperature with continuous shaking. The resulting mixture was filtered using Whatman® filter paper (number 4). The filtrate was freeze dried for 48 hours and the extract weighed to determine yield (Kaingu *et al.*, 2013).

3.3.2 DCM/Methanolic extract

One thousand eight hundred grams of *Croton menyharthii* root bark powder was weighed using a Mettler digital weighing balance (Mettler PM4600-DeltaRange®). Six fractions of the powder (300grams each) were immersed in extraction bottles each containing 800 ml of Dichloromethane: methanol (ratio of 1:1, V/V) solvent mixture and macerated for 72 hours with continuous shaking (Kenana *et al.,* 2019). The resulting mixture was filtered into a round

bottomed flask using Whatman® filter paper (number 1). A rotary evaporator was used to concentrate the filtrate *in vacuo.* Complete drying was done by placing the concentrated filtrate in a sand bath set at 50^0C for 4-7days. The extract was weighed to determine yield.

3.4 Phytochemical screening

The phytochemical components of *Croton menyharthii* root bark extracts were identified using qualitative methods.

3.4.1 Test for tannins (Ferric chloride test)

Testing for tannins was done using 0.5 g of the *Croton menyharthii* root bark aqueous extract. Ten millilitres of distilled water was added to the extract. The mixture was shaken well to mix then filtered. To two millilitres of the filtrate, a few droplets of five (5) % ferric chloride solution were added. Appearance of green, blue-green or blue-black precipitate indicated the presence of tannins. The process was repeated with the DCM/Methanolic extract (Rukenya *et al*., 2015).

3.4.2 Test for flavonoids (alkaline reagent test)

Testing for flavonoids was done using one gram of the *Croton menyharthii* root bark aqueous extract. The extract was treated with five drops of five percent (5%) sodium hydroxide solution. Two milliliters of hydrochloric acid were then added. Appearance of an intense yellow coloration which vanished on slow addition of 0.1 M hydrochloric acid indicated the presence of flavonoids. The process was repeated with the DCM/Methanolic extract (Trease and Evans, 2009).

3.4.3 Test for anthraquinones

Testing for anthraquinones was done using 3mls of *Croton menyharthii* root bark aqueous extract. Dilute Sulphuric acid was added to the extract. The resulting mixture was then boiled, filtered and allowed to cool. To the cold filtrate, an equal volume of chloroform was added and shaken to mix. The organic solvent was separated and ammonia was added. The ammoniacal

layer turning red or pink was indicative of anthraquinones presence. The process was repeated with the DCM/Methanolic extract (Rukenya *et al*., 2015).

3.4.4 Test for alkaloids

Testing for alkaloids was done using one gram of *Croton menyharthii* root bark aqueous extract. Five millilitres of one percent aqueous Hydrochloric acid was added to the extract. The resultant mixture was heated in a water bath with constant stirring and then filtered. Two millilitres of this filtrate was put in a test tube and one millilitre of dragendorrf's reagent added. Appearance of a reddish brown precipitate was considered positive for alkaloids. The process was repeated with the DCM/Methanolic extract (Rukenya *et al*., 2015).

3.4.5 Test for terpenoids

Testing for terpenoids was done using 0.2 grams of *Croton menyharthii* root bark aqueous extract. Two milliliters of acetic acid were added to the extract. The solution was cooled in a freezer after which concentrated sulfuric acid (H2SO4) was added carefully. Color variation from violet to blue or bluish green indicated presence of terpenoids. The process was repeated with the DCM/Methanolic extract (Rukenya *et al*., 2015).

3.4.6 Test for saponins (Foam test)

Testing for saponins was done using one gram of *Croton menyharthii* root bark aqueous extract. The extract was boiled with 5 ml of distilled water and then filtered. Three milliliters of distilled water were added to the filtrate and shaken forcefully for five minutes. Persistence of foam was indicative of presence of saponins. The process was repeated with the DCM/Methanolic extract (Trease and Evans, 2009).

3.4.7 Test for phenols

Testing for phenols was done using one gram of *Croton menyharthii* root bark aqueous extract. The extract was dissolved with 2ml distilled water. A few drops of 10% ferric chloride (FeCl3)

were added. Appearance of blue black or green color was indicative of presence of phenols. The process was repeated with the DCM/Methanolic extract (Trease and Evans, 2009).

3.4.8 Test for cardiac glycosides

Testing for cardiac glycosides were done using 0.5 ml of the *C. menyharthii* aqueous root bark extract One milliliter of glacial acetic acid was added to the extract. One drop of ferric chloride solution was added and the mixture shaken. One milliliter of concentrated sulfuric acid was added to the mixture. Formation of a brown ring at the interface was a positive indicator of cardiac glycosides presence. The procedure was repeated with the DCM/Methanolic extract (Rukenya *et al*., 2015).

3.5 Laboratory animals

Female wistar rats used for the research were sourced from the Department of Public Health, Pharmacology and Toxicology (PHPT), University of Nairobi. The rats were between 6-12 weeks old, non-pregnant and nulliparous, and weighed between 120-210 grams. For the acute oral toxicity study; the rats were kept in the Department of Anatomy and Physiology animal house, University of Nairobi. For the subacute toxicity study; the rats were kept in the Department of PHPT animal house. All rats were housed in cages measuring 31cm by 41cm by18cm and lined with wood shavings (a maximum of 5 rats per cage). Standard environmental condition of 12 hours artificial light and 12 hours darkness at 24-25^{o}C and a relative humidity of 56 ± 4% was maintained. The rats were acclimatized for one week before study onset. They were fed on commercially obtained pellets (Unga feeds) while tap water was provided *ad libitum*. Approval for the use of the animals was obtained from the Faculty of Veterinary Medicine Biosafety, Animal Use and Ethics Committee (BAUEC) of the University of Nairobi (Reference number FVM BAUEC/2019/185).

3.6 Acute oral toxicity testing

The acute oral toxicity study was carried out according to the Organization for Economic Cooperation and Development (OECD) test guideline 423 as described below.

The study was undertaken to determine the range of exposures where lethality is expected since death of a proportion of animals is a major end point of the test. Based on a step wise procedure, with use of a minimum number of animals per step, sufficient information was obtained on the acute oral toxicity of *Croton menyharthii*. The extract was administered orally to a group of experimental animals at one of the defined doses; each step of the procedure used 3 female rats. The absence or presence of extract related mortality of the animals at each step was used to determine the next action which would entail; no further testing; dosing of 3 other animals at same dose or dosing of 3 additional animals at the next higher or lower dose level. In case of any mortality; necropsy would be undertaken. At end of study, surviving animals would be sacrificed and necropsied.

The test was performed using the *Croton menyharthii* aqueous root bark extract. Following a randomized controlled study design, twelve rats were placed into four groups of three and labelled using picric acid for ease of identification. Food was withheld overnight before dosing but water was provided *ad libitum*. The animals' weights were taken day 0 (fasting) and just before extract dose administration. The extract administration was through intra-abdominal gavage. As per OECD guideline 423; the starting dose was 300 mg/kg (Figure 3-1). One group of 3 rats acted as the control which received one milliliter of distilled water through intra-abdominal gavage.

Food was withheld for a further four hours after administration. Special attention was given in the first four hours whereby the animals were observed keenly for the first thirty minutes and every half hour up to four hours. Each rat was observed for signs of toxicity. The signs included; changes in skin and fur colour, mucus and eye membrane, respiratory system,

circulatory system, autonomic and central nervous system and somatomotor activity. Effects like tremors, convulsions, salivations, diarrhoea, coma and death were noted. Thereafter, the animals were checked after 24 hours and daily thereafter for fourteen days.

Individual body weights of the rats were taken on day 7 and day 14. All the rats were then humanely euthanized using carbon dioxide and thereafter, necropsy was carried out. The carcasses were disposed via incineration (OECD 423, 2001).

After 24 hours observation period, with no resultant mortality, 3 rats received a dose of 2000 mg/kg body weight of the extract and observed for the first 4 hours and 24 hours thereafter. None of these rats died. Hence, a limit test was done using three rats with a dose of 5000 mg/kg body weight. The same procedure was repeated using *Croton menyharthii* DCM/methanolic extract. The control group received one milliliter of extra virgin oil (Kenana *et al*., 2019).

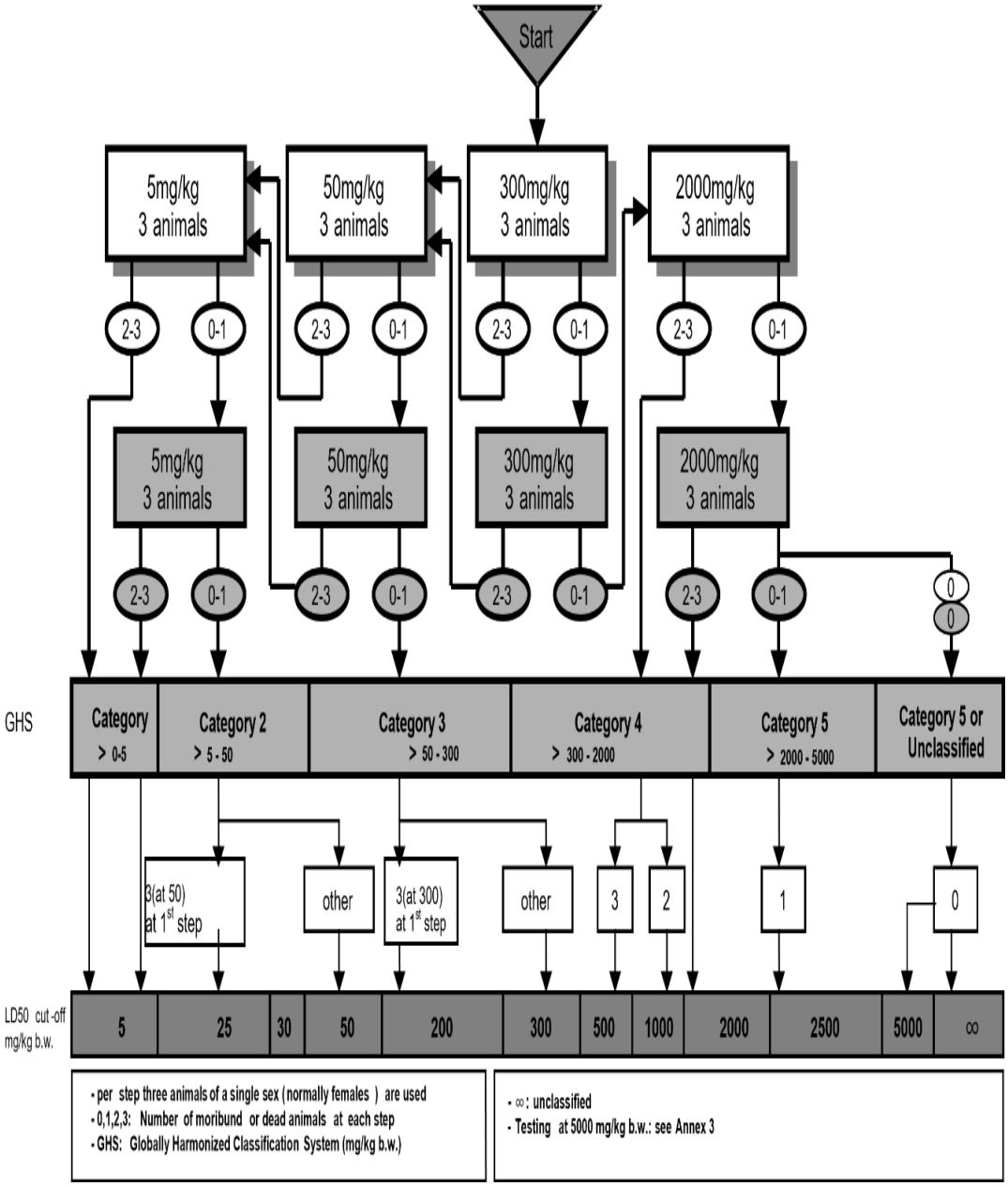

Figure 3.2: Schematic diagram of acute oral toxicity testing starting with a dose of 300 mg/kg body weight (OECD 423, 2001).

3.7 Sub-acute toxicity Testing of *Croton menyharthii* root bark extracts

3.7.1 Extract Administration

The OECD guideline 407 was used to carry out the sub-acute toxicity study as described below.

Three doses, in a two-fold descending sequence, were derived for use for both the aqueous and

the DCM/methanolic extracts of *Croton menyharthii* root barks. Determination of the doses was derived from the acute toxicity study whereby the LD_{50} for both extracts was found to be above 2000 mg/kg body weight. A high dose of 800 mg/kg body weight, an intermediate dose of 400 mg/kg body weight and a low dose of 200 mg/kg body weight were used. The dose levels were also based on a previous study (Kaingu *et al.*, 2017) where 500 and 800 mg/kg bodyweight of the aqueous extract had an antifertility effect on wistar rats but toxicity profiles of the plant was not evaluated. Using a randomized control study design, forty rats were randomly allocated into 8 groups (5 rats each) and labelled using picric acid for ease of identification.

Group 2, 3 and 4 were used as the aqueous extract test groups. Group 1 (control) received 1ml of distilled water, group 2, 3 and 4 received a daily dose of 200, 400 and 800 mg/kg body weight of extract respectively for 28 days.

Group 6, 7 and 8 were used as the organic extract test groups. Group 5 (control) received 1ml of extra virgin oil, group 6, 7 and 8 received a daily dose of 200, 400, 800 mg/kg body weight of extract respectively daily for 28 days (OECD, 2008). The gavage was carried out daily between 9 and 10 am.

3.7.2 Clinical observation

Throughout the treatment period, the animals were monitored for toxicity signs and mortality. The rats were weighed before dosing on the first day and after every seven days. Feed and water consumption for each treatment group was evaluated weekly.

3.7.3 Blood Collection

After 28 days, the rats were anaesthetized using di-ethyl ether. Approximately five to eight milliliters of blood were harvested from all rats through cardiac puncture (Kumar *et al.*, 2017). The blood was divided into two portions. For hematological analysis, the blood was put in Labex® tubes containing Ethylenediaminetetraacetic acid (EDTA) and kept at -20^0C awaiting

analysis. For biochemical analysis, the blood was put in vacutainer plain (clot activator) tubes and allowed to stand in an upright position for 30 minutes before being centrifuged at 3000 revolutions per minute to obtain serum. The serum was separated, put in Eppendorf tubes and stored at -20^{0}C awaiting biochemical analysis (Ochiai *et al.,* 2017).

3.7.4 The effects of *Croton menyharthii* root bark extracts on hematological parameters

Hematological analysis of the blood was done at Pathologists Lancet Laboratory, Mombasa Road, Nairobi. Parameters including total red blood cells count (RBC), hematocrit, mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), mean cell volume (MCV), total white blood cells count (WBC) and thrombocytes counts were determined (OECD 407, 2008).

3.7.5 The effects of *Croton menyharthii* root bark extracts on biochemical parameters

Serum biochemical analysis was performed at Pathologists Lancet Laboratory, Mombasa Road, Nairobi. Biochemical parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total protein. The parameters were used to evaluate the effect of the extract on liver function, whereas blood urea nitrogen (BUN) and creatinine were determined to evaluate the effect of the extract on kidney function (OECD 407, 2008).

3.7.6 Pathological changes caused by exposure to *Croton menyharthii* root bark extracts

After blood harvesting, the animals were humanely euthanized and post-mortem examination performed on all rats. Internal organs were examined for gross pathological changes. The liver and the kidneys were harvested, washed with physiological saline and their wet weights were taken using a Mettler digital weighing balance (Mettler PM4600-DeltaRange®). Their relative organ-body weight ratios were calculated using the formula below.

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight}}{\text{Weight of the animal}} \times 100$$

They were then fixed in 10% formalin and processed for histopathological examination. The tissues were trimmed, embedded in paraffin wax and then sectioned using a microtone (Leitz wetzlar®) at 5 μm. The tissue blocks were then stained using haematoxylin and eosin (H&E). A light microscope (Leica DM 500®) was used to observe them using objective lenses of 40X, 100X and 400X magnification (Slaoui and Fiette, 2011).

3.8 Statistical analysis

The weights, biochemical and hematological parameters were expressed as mean ± standard deviation of the mean. One-way analysis of variance (ANOVA) was used to analyze the means using IBM SPSS Statistics software version 21a. To find out whether there was a significant difference among the groups and the controls, a Tukey post hoc test was performed (Kenana *et al.*, 2019). Differences were considered statistically significant at $P < 0.05$.

CHAPTER FOUR
RESULTS

4.1 Extract yield

Aqueous *Croton menyharthii* root barks extract yield was 81.25 grams (6.25%). The extract was a dark brown powder that was highly water soluble. DCM-methanolic *C. menyharthii* root barks extract yield was 54.54 grams (3.03%). It was a tar like substance, dark-brown in color and sparingly soluble in water. It was however soluble in extra virgin oil after warming in a water bath at 50^{o}C and triturating in a mortar with a pestle to form a fine suspension.

4.2 Phytochemical composition

Both *C. menyharthii* aqueous and DCM-methanolic extract root bark extracts tested positive for alkaloids, saponins, tannins, phenols and cardiac glycosides. Both extracts tested negative for anthraquinones, flavonoids and terpenoids (Table 4-1).

Table 4.1: Phytochemical composition of aqueous and DCM-Methanolic *C. menyharthii* root bark extracts.

Phytochemical compound	Aqueous extract	DCM-methanolic extract
Tannins	+	+
Flavonoids	-	-
Anthraquinones	-	-
Alkaloids	+	+
Terpenoids	-	-
Saponins	+	+
Phenols	+	+
Cardiac glycosides	+	+

Key: + =present; - = absent

4.3 Acute Oral toxicity test of *Croton menyharthii* root bark extracts

4.3.1 *Croton menyharthii* root bark extracts effects on clinical and physical behaviors of rats in acute oral toxicity study

Both *Croton menyharthii* aqueous and DCM-methanolic extract single dose of 300 mg/kg body weight did not cause mortality, adverse physical or clinical effects. Immediately after dosing, all rats exhibited increased respiration, followed by drowsiness and lethargy within the first 30 minutes. The rats recovered within two hours.

On increasing to 2000 mg/kg body weight dose of both extracts, none of the animals died. The animals also exhibited increased respiration immediately after dosing, followed by drowsiness and lethargy which cleared within four hours. No adverse effects were observed. The limit dose of 5000 mg/kg did not result in noticeable significant signs of toxicity. The rats that received the DCM/methanolic extract groomed and rubbed their mouth against the walls of the cages for approximately ten minutes after dosing.

For the rest of the study period, the rats exhibited no signs of toxicity and none was moribund or died (Table 4.2). This led to the conclusion that the LD_{50} values for both the aqueous and DCM/methanolic extract of *C. menyharthii* root barks are >2000 mg/kg body weight.

Table 4.2 : Effect of single dose of *C. menyharthii* aqueous and DCM-Methanolic root bark extracts on rats' physical and behavioral parameters of female rats.

Observation	Control 1ml distilled water	Control 1ml extra virgin oil	Aqueous 300 mg/kg	DCM-Methanolic 300mg/kg	Aqueous 2000 mg/kg	DCM-Methanolic 2000mg/kg	Aqueous 5000 mg/Kg	DCM-Methanolic 5000mg/Kg
Respiratory changes	-	-	+	+	+	+	+	+
Circulatory changes	-	-	-	-	-	-	-	-
Skin/fur changes	-	-	-	-	-	-	-	-
Eye color changes	-	-	-	-	-	-	-	-
Gripping strength	+	+	+	+	+	+	+	+
Sound response	+	+	+	+	+	+	+	+
Response to touch	+	+	+	+	+	+	+	+
Locomotion	+	+	+	+	+	+	+	+
Urination	+	+	+	+	+	+	+	+
Defaecation	+	+	+	+	+	+	+	+
Diarrhoea	-	-	-	-	-	-	-	-
Righting reflex	+	+	+	+	+	+	+	+
Lethargy	-	-	+	+	+	+	+	+
Sedation/ Drowsiness	-	-	+	+	+	+	+	+
Tremors	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-
Mortality	-	-	-	-	-	-	-	-
Gross necropsy	No change	No Change	No change	No change	No change	No change	No change	No change

4.3.2 The effect of the *C. menyharthii* root bark extracts on weekly mean body weights of rats in acute oral toxicity study

a) C. menyharthii aqueous root barks extract effect on weekly mean body weights of rats in acute oral toxicity study

There was an increase in mean body weight of rats in all the *C. menyharthii* aqueous root bark extract treatment groups as well as the control group over the 14 days period of acute oral toxicity study. At 300 and 2000 mg/kg body weight, the weight gain was non-significant ($P>0.05$) compared to the control. However, at 5000 mg/kg there was a significance low weight gain ($P<0.013$) in the treatment group when compared to the control. As the treatment dose increased, percentage weight gain reduced (Table 4.3).

Table 4.3: *Croton meny* *harthii* aqueous root bark extract single dose effects on weekly mean body weights of rats

Dose group	Initial weight	Day 7	Day 14	%Weight increase	P-value
Control	137.25 ± 30.57	169.38 ± 30.07	192.68 ± 29.92	40.39	-
300 mg/kg Bwt	183.91 ± 14.86	212.02 ± 16.25	230.94 ± 17.12	25.57	0.178
2000 mg/kg Bwt	169.99 ± 11.89	197.73 ± 13.41	209.04 ± 13.26	22.97	0.536
5000 mg/kg Bwt	225.02 ± 14.94	244.74 ± 16.24	260.47 ± 14.39	15.75	0.013

Values are expressed as mean ± standard deviation. Significant difference ($P \leq 0.05$) (n=3)

b) C. menyharthii DCM/Methanolic extract root barks extract effect on weekly mean body weights of rats in acute oral toxicity study

There was an increase in mean body weight of rats in all the treatment groups of *Croton menyharthii* DCM/Methanolic extract as well as the control group over the 14 days. The increase in weight in the treatment groups was non-significant (P>0.05) when compared to the control. The highest weight gain was observed in the 300 mg/kg body weight treatment group (Table 4.4).

Table 4.4: *C. menyharthii* DCM/Methanolic root bark extract single dose effects on weekly mean body weight of rats

Extract dose	Initial weight	Day 7	Day 14	%Wt increase	P-value
Control	152.88 ± 14.88	180.28 ± 17.06	208.26 ± 17.38	36.22	-
300 mg/kg Bwt	168.84 ± 14.49	196.42 ± 13.09	239.83 ± 17.12	42.05	0.848
2000 mg/kg Bwt	165.39 ± 6.53	195.55 ± 6.21	226.62 ± 1.58	34.60	0.933
5000 mg/kg Bwt	181.75 ± 5.95	210.67 ± 5.80	248.89 ± 6.93	36.94	0.604

Values are expressed as mean ± standard deviation Significant difference ($p \leq 0.05$) (n=3)

4.3.3 Gross pathology

There were no pathological changes of major organs (liver, kidneys, spleen, heart, lungs, stomach, small and large intestines) of rats treated with *C. menyharthii* aqueous and DCM-Methanolic extracts as well as the control groups on macroscopic examination. There were no abnormalities, necrosis, inflammation or changes in size or colour.

4.4 Sub-acute toxicity study of *Croton menyharthii* root bark extracts

4.4.1 Effects of *C. menyharthii* root bark extract on physical and clinical behaviour of rats in sub-acute toxicity study

Rats in both aqueous and DCM-Methanolic *Croton menyharthii* extracts treatment groups appeared normal clinically and physically during and after the 28-days daily administration even at the highest dose (800mg/kg body weight). However, all rats that received the DCM/Methanolic extract groomed themselves around their mouths a few minutes after dose administration. They also were rubbing their mouths against the walls and the floor of their cages indicating some form of irritation. There was however no mortality in any of the treatment groups.

4.4.2 *Croton menyharthii* root bark extracts effects on feed consumption of rats in sub-acute toxicity study

There was a significant difference ($P<0.016$) on feed consumption at 400 mg/kg body weight *Croton menyharthii* aqueous root bark extract between the treatment group and the control. However, there was no significant difference ($P>0.05$) in feed consumption at 200 and 800 mg/kg body weight *Croton menyharthii* aqueous root bark extract between the treatment groups and the control.

At 200 mg/kg body weight dose of *C. menyharthii* DCM/Methanolic root barks extract, there was a significant difference ($P<0.033$) in feed consumption between the treatment group and the control. However, there was non-significant difference ($P>0.05$) in feed consumption for the 400 and 800 mg/kg body weight treatment groups when compared to the control (Table 4.5).

Table 4.5: The effects of *Croton menyharthii* root bark aqueous and DCM/Methanolic extracts graded doses on average weekly feed consumption of rats.

Weekly feed consumption for aqueous extract					
Dose	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**p-value**
Control	109.73±16.15	101.67±6.53	101.44±11.56	96.86±22.46	-
200 mg/kg Bwt	112.70±19.79	105.33±21.95	112.69±11.01	112.67±12.29	0.282
400 mg/kg Bwt	111.32±23.69	128.11±8.30	122.55±12.40	112.12±23.73	0.016
800 mg/kg Bwt	96.76±40.67	110.10±4.46	108.50±10.93	112.65±16.59	0.737
Weekly feed consumption for DCM/Methanolic extract					
Control	84.87±24.47	76.97±5.83	75.91±9.15	73.43±6.09	-
200 mg/kg Bwt	98.63±13.84	93.06±6.91	93.15±7.97	94.03±17.58	0.033
400 mg/kg Bwt	90.98±15.79	82.57±3.80	73.65±12.45	75.14±22.56	0.950
800 mg/kg Bwt	97.70±16.12	75.94±3.59	75.65±11.07	74.12±15.10	0.936

Values are recorded in grams per dose group per day and expressed as mean ± standard deviation. Significant difference ($P \leq 0.05$) (n=5)

The *C. menyharthii* DCM/Methanolic root barks extract treatment and control groups exhibited a gradual reduction in the amounts of feed consumed over the weeks. The rats in the *C. menyharthii* aqueous root bark extract 400mg/kg body weight treatment group had the highest feed consumption. Feed consumption among the aqueous *C. menyharthii* root barks extract treatment groups rats was generally higher than their respective controls; and than those of the DCM/Methanolic extract treatment groups (Figure 4.1).

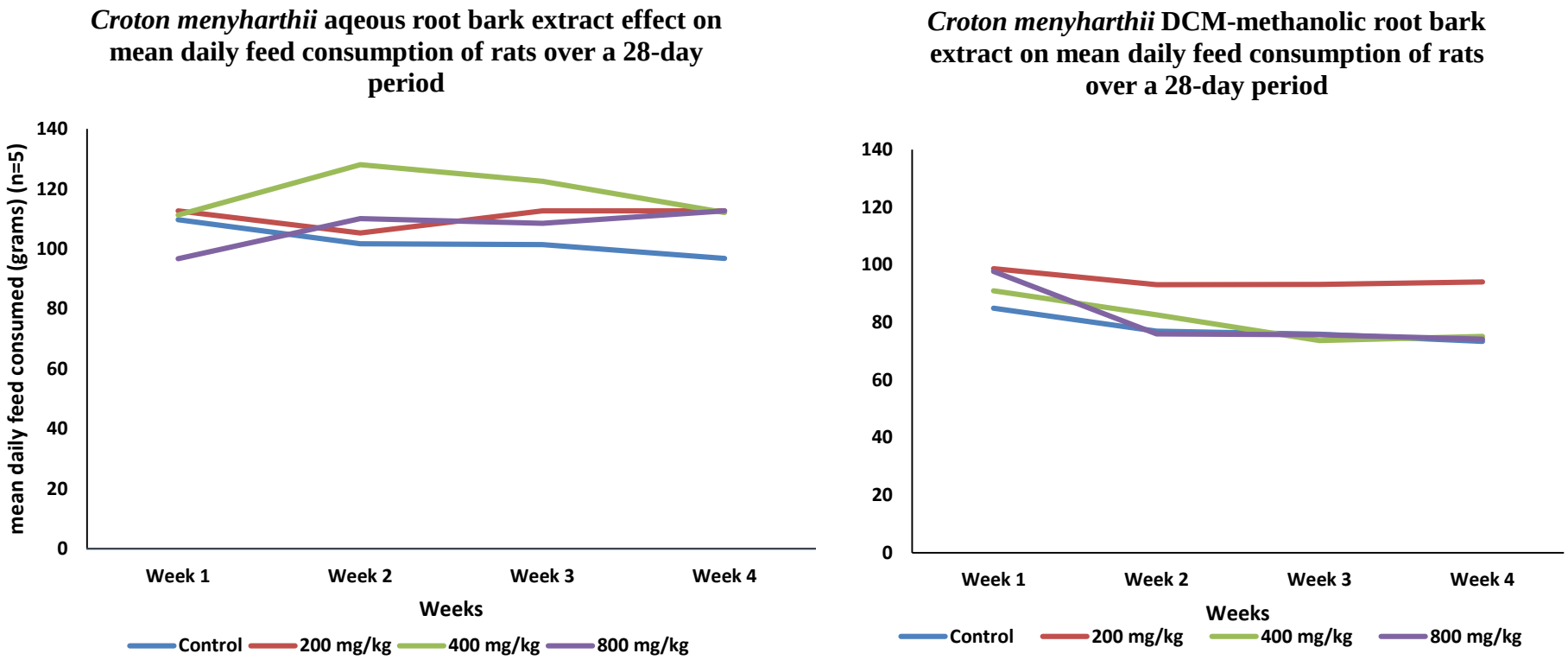

Figure 4.1 : *Croton menyharthii* aqueous and DCM/Methanolic root bark extracts graded doses on rat feed consumption over a 28-days period.

4.4.3 *Croton menyharthii* effects on average water consumption of rats in sub-acute toxicity study

There was a significant difference (P<0.040; P<0.03) in water consumption for the rats in the *C. menyharthii* 200 and 400 mg/kg body weight aqueous root barks extract treatment groups respectively when compared to the control. There was no significant difference (P>0.05) between the 800mg/kg body weight treatment group and the control.

There was a non-significant difference (P>0.05) in water consumption for the rats in all the DCM/Methanolic extract treatment groups when compared to the control group (Table 4.6).

Table 4.6: The effect *Croton menyharthii* root bark aqueous and DCM/Methanolic extracts graded doses on average weekly water consumption of rats.

Weekly water consumption for aqueous extract					
Dose	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**P-value**
Control	139.28±26.37	129.29±11.34	148.57±13.76	141.43±55.67	-
200 mg/kg Bwt	161.43±21.74	160.71±24.23	153.57±39.02	168.57±48.71	0.040
400 mg/kg Bwt	155.71±38.13	187.86±18.68	168.57±38.37	175.71±48.60	0.003
800 mg/kg Bwt	143.57±50.23	156.43±22.86	160.00±40.52	167.86±38.28	0.111
Weekly water consumption for DCM/Methanolic extract					
Control	129.29±66.61	137.14±31.60	129.29±7.32	125.71±38.88	-
200 mg/kg Bwt	147.14±18.90	158.57±11.80	164.29±22.81	147.14±43.96	0.100
400 mg/kg Bwt	142.14±17.04	135.71±10.58	125.00±21.41	117.14±46.45	1.000
800 mg/kg Bwt	140.71±22.44	123.57±11.80	142.86±12.86	131.43±49.05	0.895

Values are recorded in milliliters per dose group per day and expressed as mean ± standard deviation. Significant difference ($p \leq 0.05$) (n=5)

Water consumption among rats in the *C. menyharthii* DCM/Methanolic root barks extract treatment groups reduced with time and was less compared to the aqueous extract treatment groups (Figure 4.2).

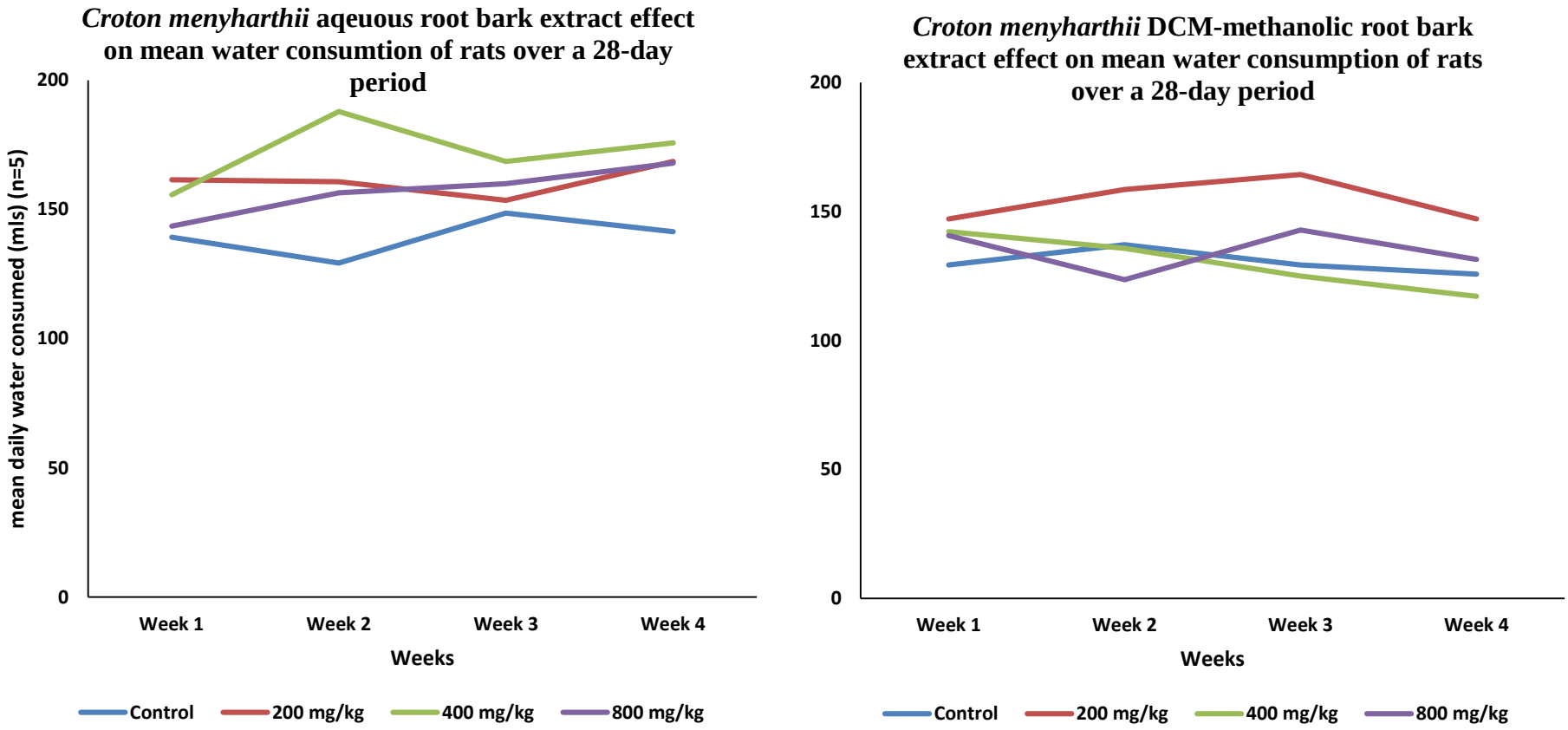

Figure 4.2: Effects of *Croton menyharthii* aqueous and DCM/Methanolic root bark extracts graded doses on water consumption in rats over a 28-days period.

4.4.4 *Croton menyharthii* effects on mean weekly body weights of rats in sub-acute toxicity study

Both *C. menyharthii* aqueous and DCM/Methanolic extract treatment groups had a non-significant (P>0.05) increased weight gain than their respective controls. The *C. menyharthii* aqueous control group had an overall mean weight gain of 21.82%. The 200, 400, and 800 mg/kg body weight aqueous treatment groups had an average weight gain of 29.24, 36.35 and 26.47% respectively over the twenty eight days.

The *C. menyharthii* DCM/Methanolic control group had an overall mean weight gain of 20.28%. The 200, 400, and 800 mg/kg body weight DCM/Methanolic treatment groups had an average weight gain of 23.15, 27.89 and 32.16% respectively over the twenty eight days (Table 4.7). All treatment groups had a non-significant increase in mean weight when compared to their respective controls.

Table 4.7: *C. menyharthii* aqueous and DCM/Methanolic root bark extract graded doses effects on mean weekly body weights of rats.

Mean weekly body weights of rats treated with aqueous extract of *Croton menyharthii* root barks extracts for 28 days						
Dose	**Day 0**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**p-value**
Control	188.90 ± 14.28	221.38 ± 22.20	223.30 ± 26.60	225.19± 25.48	230.11 ± 26.63	-
200 mg/kg	176.23 ± 12.17	203.95 ± 10.29	198.85 ± 8.17	208.67 ± 10.56	227.76 ± 14.90	0.620
400 mg/kg	169.23 ± 12.81	198.91 ± 11.66	214.08 ± 8.71	217.72 ± 8.44	230.74 ± 9.56	0.767
800 mg/kg	169.41 ± 12.22	189.81 ±14.18	196.84 ± 13.84	198.73 ± 16.98	214.26 ± 16.31	0.229
Mean weekly body weights of rats treated with DCM/Methanolic extract of *Croton menyharthii* root barks extracts for 28 days						
Control	183.47 ± 15.76	199.42 ± 21.24	209.13 ± 24.64	215.71 ± 27.21	220.68 ± 30.44	-
200 mg/kg	178.90 ± 13.14	199.70 ± 16.60	207.70 ± 18.62	211.23 ± 18.26	220.31 ± 18.04	0.997
400 mg/kg	178.29 ± 14.28	205.97 ± 19.14	215.31 ± 17.26	220.67 ± 16.26	228.02 ± 18.96	0.984
800 mg/kg	164.16 ± 8.38	188.28 ± 11.51	200.42 ± 13.84	206.46 ± 14.83	216.96 ± 14.21	0.797

Values are in grams and expressed as mean ± standard deviation; Significant difference

($P \leq 0.05$) (n=5)

There was a sharp body weight gain in the first one week in all the treatment groups and their respective controls. This was followed by a gradual increase over the next three weeks (Figure 4.3).

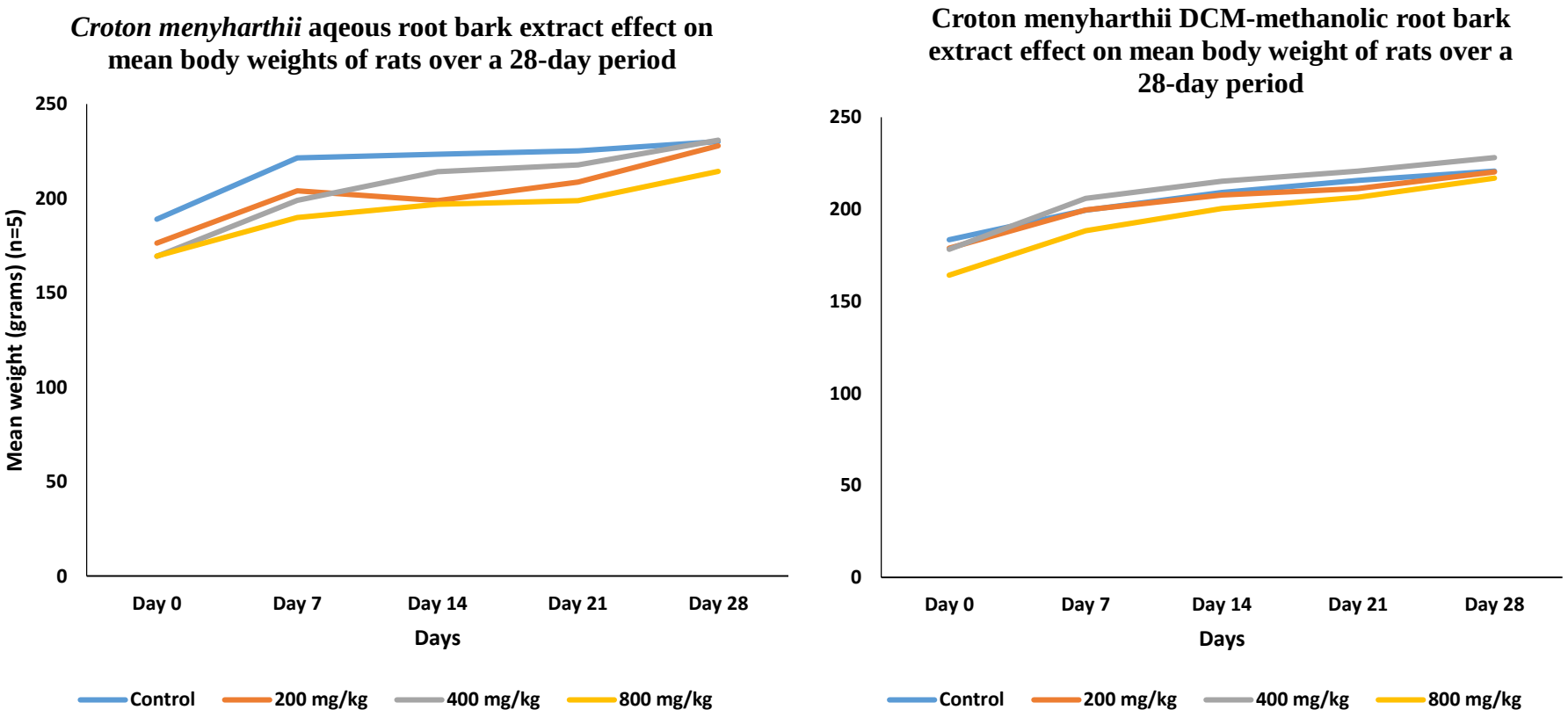

Figure 4.3: *C. menyharthii* aqueous and DCM/Methanolic root barks graded doses effects on mean weekly body weights of rats over a 28-day period

4.4.5 Effects of *Croton menyharthii* root bark extracts on hematological parameters of rats in sub-acute toxicity study

There was non-significant difference (P>0.05) in all haematological parameters across all *C. menyharthii* aqueous treatment groups when compared to the control (Table 4.8).

Table 4.8: The effect of *C. menyharthii* aqueous root barks extract on haematological parameters in female rats after 28 days oral administration

Parameter	Control	200mg/kg	*P value*	400mg/kg	*p value*	800mg/kg	*P Value*
RBC (x 10^6/µL)	7.47± 1.03	7.76±1.04	***0.098***	7.37±1.03	***0.988***	7.12± 1.56	***0.979***
WBC (x10^3/µL)	9.50± 1.05	11.71±5.70	***0.829***	12.84± 1.92	***0.587***	14.17± 3.98	***0.318***
Platelets (x10^3/µL)	682.00± 180.53	875.00± 188.49	***0.315***	769.00± 128.78	***0.617***	714.00± 78.84	***0.617***
Hb (gm/dL)	14.10± 2.57	14.00±1.55	***1.00***	14.00± 1.27	***1.00***	13.60± 3.09	***0.992***
PCV (%)	42.3± 7.89	41.1 ±4.87	***0.993***	41.5 ± 4.36	***0.998***	40.4± 9.32	***0.978***
MCV (fL)	56.60± 2.14	53.10±3.68	***0.263***	56.5±2.65	***1.00***	56.60± 0.96	***1.00***
MCH (pg)	18.80± 0.50	18.00±0.75	***0.498***	19.10± 1.00	***0.942***	19.10± 0.70	***0.955***
MCHC (g/dL)	33.20± 0.53	34.00±1.51	***0.690***	33.80± 0.66	***0.842***	33.70± 0.94	***0.888***

RBC: Red blood cells, WBC: White blood cells, **Hb:** Haemoglobin, **PCV:** Packed cell volume, **MCV:** Mean corpuscular volume, **MCH:** Mean corpuscular haemoglobin, **MCHC:** Mean corpuscular haemoglobin concentration. Values expressed as mean ± SD (n=5). Significant difference (P≤0.05)

There was dose dependent increase in WBC Counts, and a non-dose dependent increase in the platelets levels. There was also dose dependent decrease in RBCs, Hb and PCV levels (Figure 4.4). However, the variations were non-significant (P>0.05).

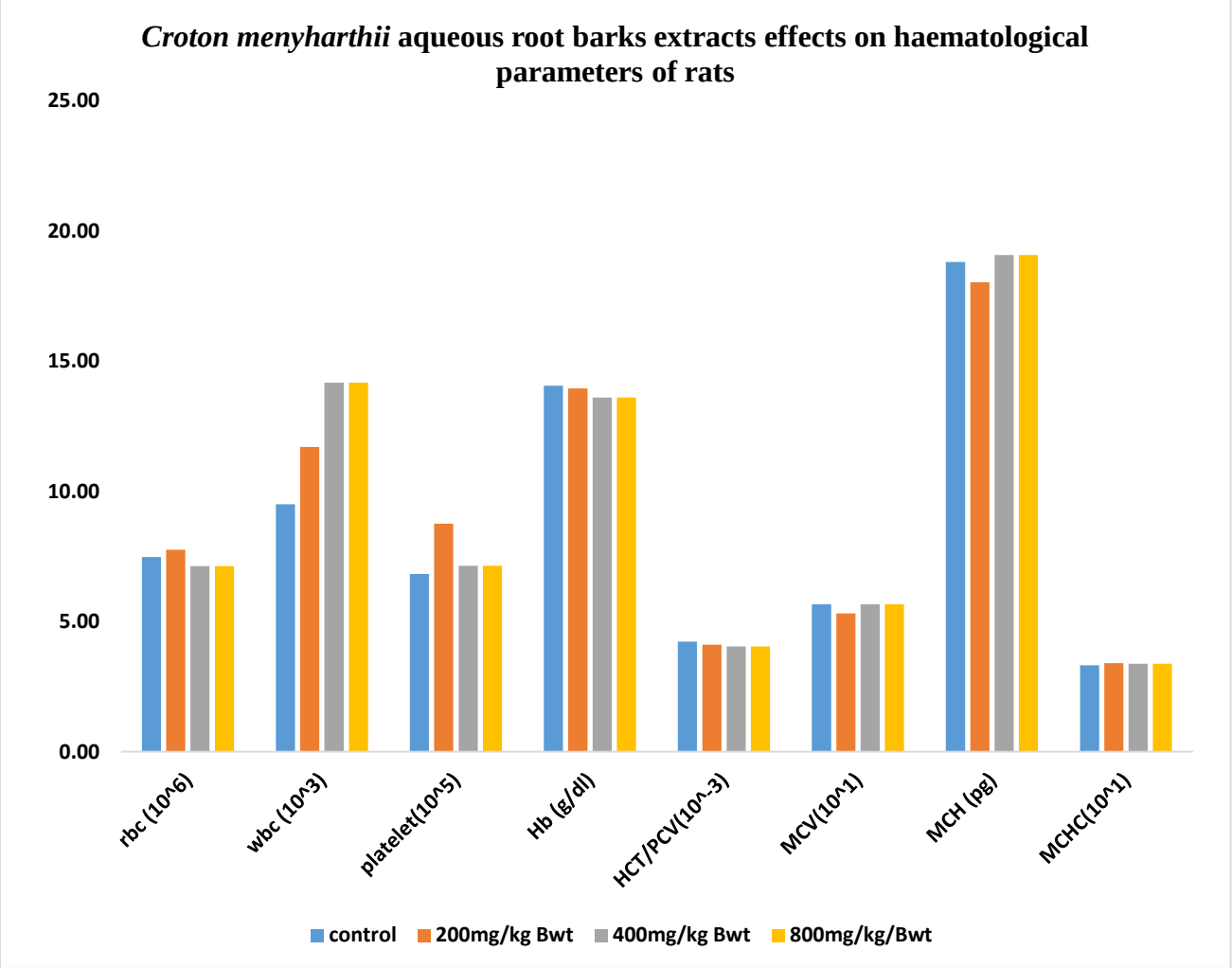

Figure 4.4: Effects of *Croton menyharthii* aqueous root barks extract on haematological parameters of rats after 28 days daily oral administration.

There was non-significant difference (P >0.05) in all the haematological parameters in the *Croton menyharthii* DCM/Methanolic root barks extract treatment groups when compared to the control (Table 4.9).

Table 4.9: *C. menyharthii* DCM/Methanolic root barks extract effects on haematological parameters of female rats after daily administration for 28 days.

Parameter	Control	200mg/kg	*p value*	400mg/kg	*p value*	800mg/kg	*P value*
RBC (x 10^6/µL)	7.38± 0.77	6.89±1.64	***0.883***	8.18±0.48	***0.638***	8.25±0.24	***0.576***
WBC (x10^3/µL)	11.73± 1.86	11.65±2.28	***1.00***	14.48±1.92	***0.175***	9.38±1.89	***0.281***
Platelets (x10^3/µL)	513.00± 342.43	696.00± 76.64	***0.574***	582.25±11 6.99	***0.959***	769.00±13 8.48	***0.306***
Hb (gm/dL)	14.30± 1.79	13.20±3.12	***0.851***	15.20±0.65	***0.898***	15.00±0.95	***0.936***
PCV	41.8± 5.07	39.30±8.56	***0.896***	44.3±1.33	***0.899***	43.90±1.51	***0.939***
MCV (fL)	56.60± 3.04	57.40±1.64	***0.962***	54.20±1.81	***0.451***	53.20±2.12	***0.183***
MCH (pg)	19.30± 1.01	19.10±0.53	***0.990***	18.60±0.44	***0.578***	18.20±1.07	***0.273***
MCHC (g/dL)	34.10± 0.62	33.40±1.07	***0.698***	34.20±0.49	***0.995***	34.30±1.21	***0.992***

RBC: Red blood cells, WBC: White blood cells, **Hb:** Haemoglobin, **PCV:** Packed cell volume, **MCV:** Mean corpuscular volume, **MCH:** Mean corpuscular haemoglobin, **MCHC:** Mean corpuscular haemoglobin concentration. Values expressed as mean ± SD Significant difference (P≤0.05) (n=5).

There was a dose dependent increase in the number of RBCs and Hb; and a non-dose dependent increase in the platelets levels. There was also a dose dependent decrease in MCV and MCH (Figure 4.5). The variations were however non-significant (Table 4.9).

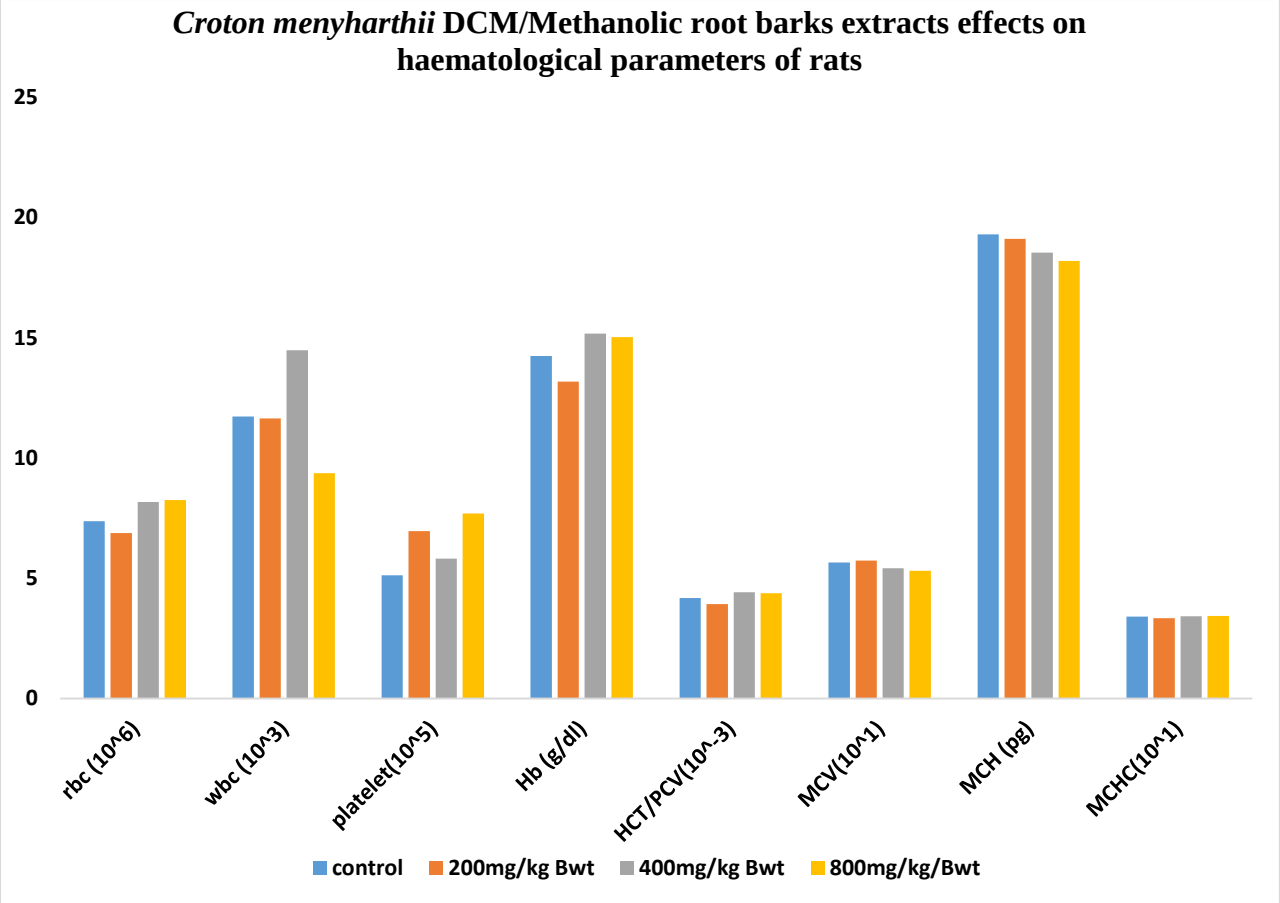

Figure 4.5: *C. menyharthii* DCM/Methanolic root barks extract effects on haematological parameters of female rats after 28 days oral daily administration.

4.4.6 The effect of *Croton menyharthii* root bark extracts on biochemical parameters in sub-acute toxicity study

There was significant (P< 0.05) low total serum proteins levels across all *C. menyharthii* aqueous root barks extract treatment groups when compared to the control. However, there was a non- significant (P > 0.05) difference in serum levels of ALT, AST, BUN and creatinine across all *C. menyharthii* root barks aqueous treatment groups when compared to the control (Table 4.10).

Table 4.10: *C. menyharthii* aqueous root barks extract effects on female rats biochemical parameters after a 28-day graded dose oral administration

Parameter	Control	200mg/kg	*p value*	400mg/kg	*p value*	800mg/kg	*p value*
ALT mg/l	118.00± 13.89	119.00±30. 23	***1.000***	136.00± 23.34	***0.681***	109.00± 21.50	***0.955***
AST ml/l	219.00± 46.41	202.00±27. 80	***0.976***	241.00± 103.57	***0.950***	153.00± 18.91	***0.428***
T.P g/l	87.00± 6.86	75.00±2.55	***0.006***	69.00±3.42	***0.000***	69.50±3.54	***0.000***
BUN	8.00± 1.00	9.00±0.25	***0.980***	8.00±0.45	***0.898***	7. 00±1.72	***0.445***
Creatinine mg/dl	47.00± 2.24	52.00±5.88	***0.971***	63.00± 29.47	***0.526***	47.00±5.73	***1.000***

ALT: Alanine aminotransferase, **AST:** Aspartate amino transferase, **TP:** Total protein, **BUN:** Blood urea nitrogen. Values are expressed as mean ± SD Significant difference (P≤0.05) (n=5).

There was a significant dose-dependent decrease in serum total proteins in the aqueous *C. menyharthii* root barks extract treatment groups when compared to the control. There was non-dose dependent rise in serum creatinine levels in the aqueous *C. menyharthii* root barks extract treatment groups which was non-significant when compared to the control. The 400 mg/kg body weight treatment group had the highest level of ALT and AST (Figure 4.6)

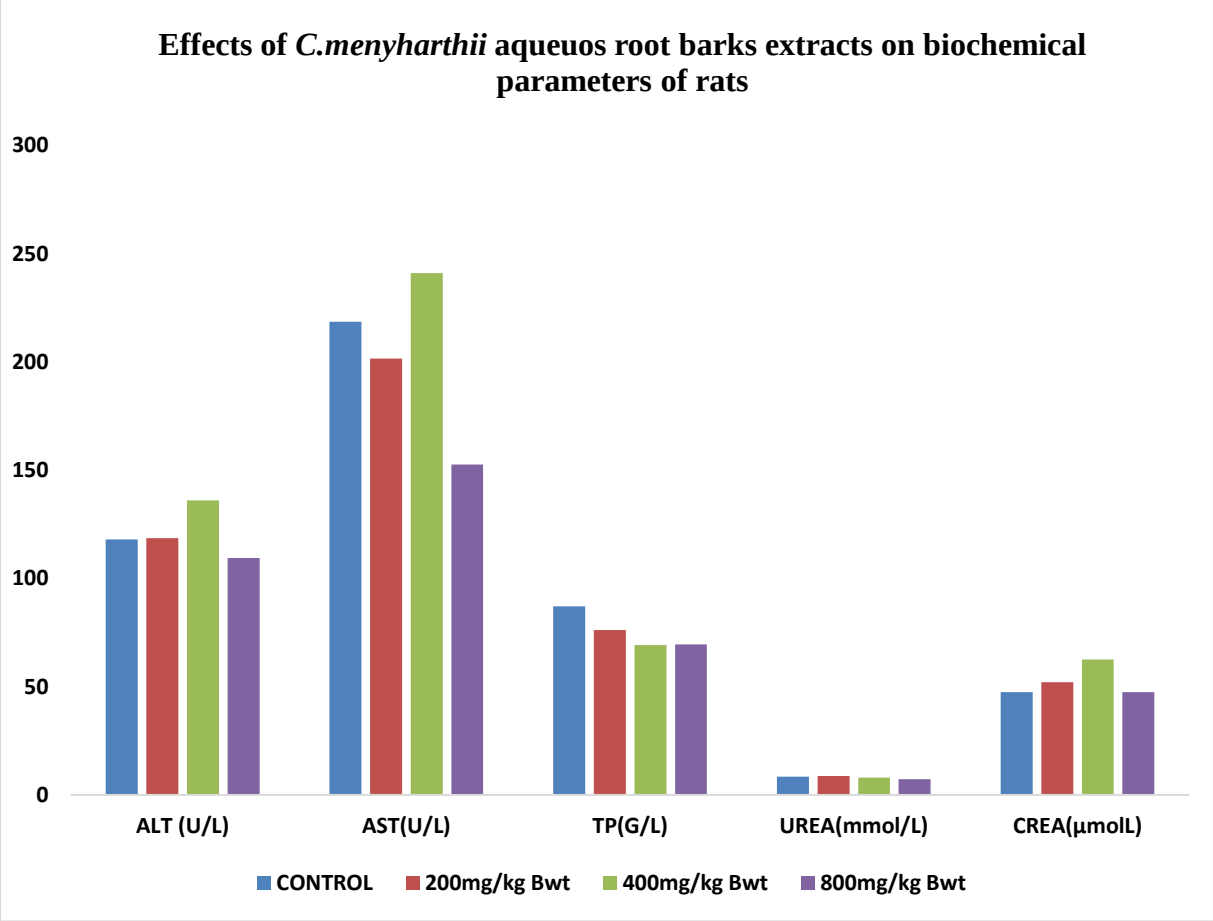

Figure 4.6: *C. menyharthii* aqueous root barks extract effects on biochemical parameters of female rats after 28 days graded dose oral administration

In the *C. menyharthii* DCM/Methanolic root barks extract treatment groups, there was non-significant difference ($P > 0.05$) in all biochemical parameters across all dose groups when compared to the control group (Table 4.11).

Table 4.11: *C. menyharthii* DCM/Methanolic root barks extract effects on biochemical parameters in female rats after oral administration with graded doses for 28 days.

Parameter	Control	200mg/kg	*p value*	400mg/kg	*p value*	800mg/kg	*p value*
ALT mg/l	111.00± 31.05	107.00± 22.05	***0.990***	133.00± 28.95	***0.583***	108.00± 11.63	***0.999***
AST ml/l	191.00± 66.42	162.00± 8.50	***0.851***	185.00± 34.32	***0.997***	164.00± 42.32	***0.820***
T.P g/l	75.00± 2.62	75.00±4.59	***0.961***	75.00± 2.16	***0.997***	70.00±4.21	***0.304***
BUN	8.00±1.00	9.00±0.25	***0.980***	8.00±0.45	***0.898***	7.00±1.72	***0.445***
Creatinine mg/dl	46.00± 3.78	43.00±2.32	***0.993***	45.00± 4.38	***0.987***	47.00± 11.68	***0.998***

ALT: Alanine aminotransferase, **AST:** Aspartate amino transferase, **TP:** Total protein, **BUN:** Blood urea nitrogen. Values are expressed as mean ± SD Significant difference ($P \leq 0.05$) (n=5).

In all the *C. menyharthii* DCM/Methanolic root barks extract treatment groups, total proteins levels decreased in a dose dependent manner whereas, AST and BUN levels decreased in a non-dose dependent manner. The variations were non-significant when compared to the control. The 400 mg/kg body weight treatment group recorded the highest levels of ALT and AST (Figure 4.7).

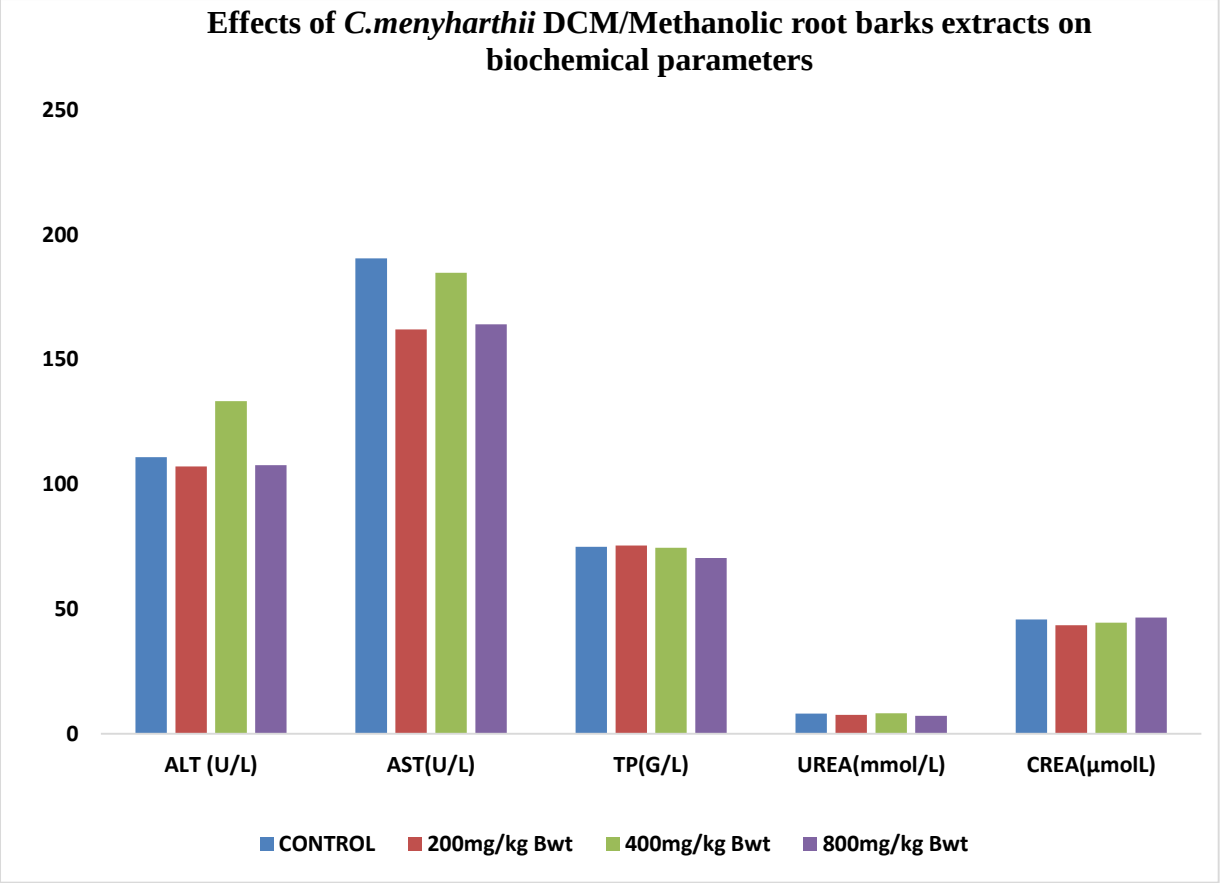

Figure 4.7: *C. menyharthii* DCM/Methanolic root barks extract effects on biochemical parameters of female rats after oral administration with graded doses for 28 days

4.4.7 Gross pathology

On macroscopic examinations, there was no pathological difference on major organs of rats in all the *C. menyharthii* aqueous and DCM/Methanolic root barks extract treatment groups when compared to the control. There was neither inflammation and necrosis nor changes in size and colour of the heart, lungs, liver, stomach, small and large intestines, spleen and kidneys.

4.4.8 Relative-Organ Weight Ratios

There was a significant difference ($P<0.045$) in the mean relative-organ weight ratio of the liver for the rats in the 200 mg/kg bodyweight *C. menyharthii* DCM/methanolic extract treatment group. There were no other significant differences ($P>0.05$) in organ-body weight ratios of liver and kidneys for the rest of the treatment groups for both *C. menyharthii* aqueous

and DCM/Methanolic root barks extract when compared to their respective controls (Table 4.12).

Table 4.12: *C. menyharthii* aqueous and DCM/Methanolic extract effects on relative organ-body weight ratios in rats after 28 days graded dose oral administration

Aqueous extract effects on Relative organ- body weight ratios						
Dose	**Liver**	**P-value**	**Left kidney**	**P-value**	**Right kidney**	**P- value**
Control	0.03700 ± 0.00399	-	0.003746 ± 0.000388	-	0.003845 ± 0.000466	-
200	0.03802 ± 0.00380	0.969	0.00373 ± 0.000250	1.000	0.003935 ± 0.000278	0.957
400	0.03919 ± 0.00174	0.770	0.003628 ± 0.000269	0.897	0.003794 ± 0.000172	0.992
800	0.03797 ± 0.00469	0.211	0.003696 ± 0.000196	0.997	0.003874 ± 0.000224	0.558
DCM/Methanolic extract effects on Relative organ- body weight ratios						
Control	0.03825 ± 0.002301	-	0.003817 ±0.000462	-	0.004084 ± 0.000372	-
200	0.03156 ± 0.002749	0.045	0.003885 ± 0.000442	0.991	0.003643 ± 0.000232	0.174
400	0.03523 ± 0.005741	0.563	0.003546 ± 0.000270	0.668	0.003505 ± 0.000351	0.051
800	0.03361 ± 0.002586	0.220	0.003977 ± 0.000282	0.906	0.003773 ± 0.000311	0.443

Values are expressed as mean ± SD Significant difference ($P \leq 0.05$) (n=5).

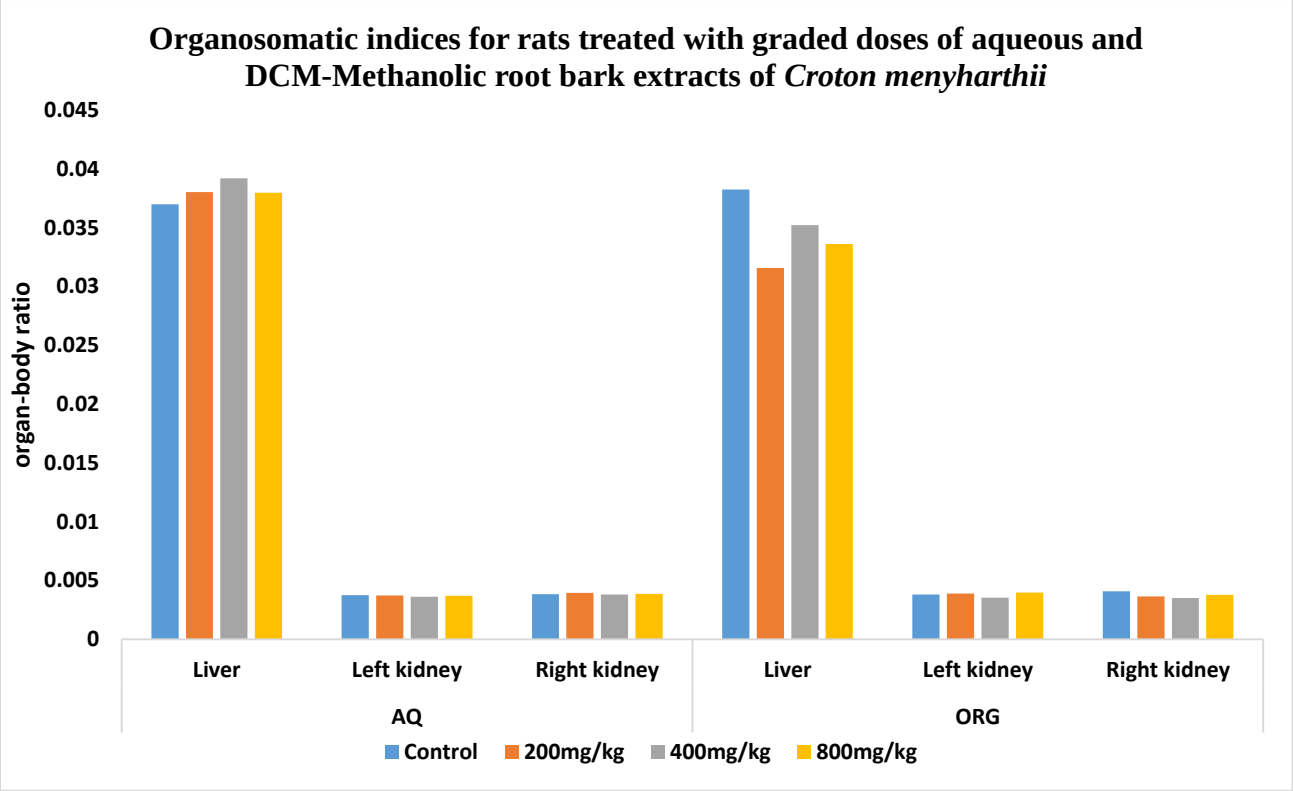

Figure 4.8: *C. menyharthii* aqueous and DCM/Methanolic root barks extracts effects on relative organ- body weight ratios of rats after daily oral administration with graded doses for 28 days.

4.4.9 Histopathological findings in sub-acute toxicity of *Croton menyharthii* root bark extracts

a) Effects of *Croton menyharthii* aqueous root bark extracts in the Liver

There was mild congestion of portal blood vessels in all *Croton menyharthii* aqueous root bark extract treatment groups. (Figure 4.9).

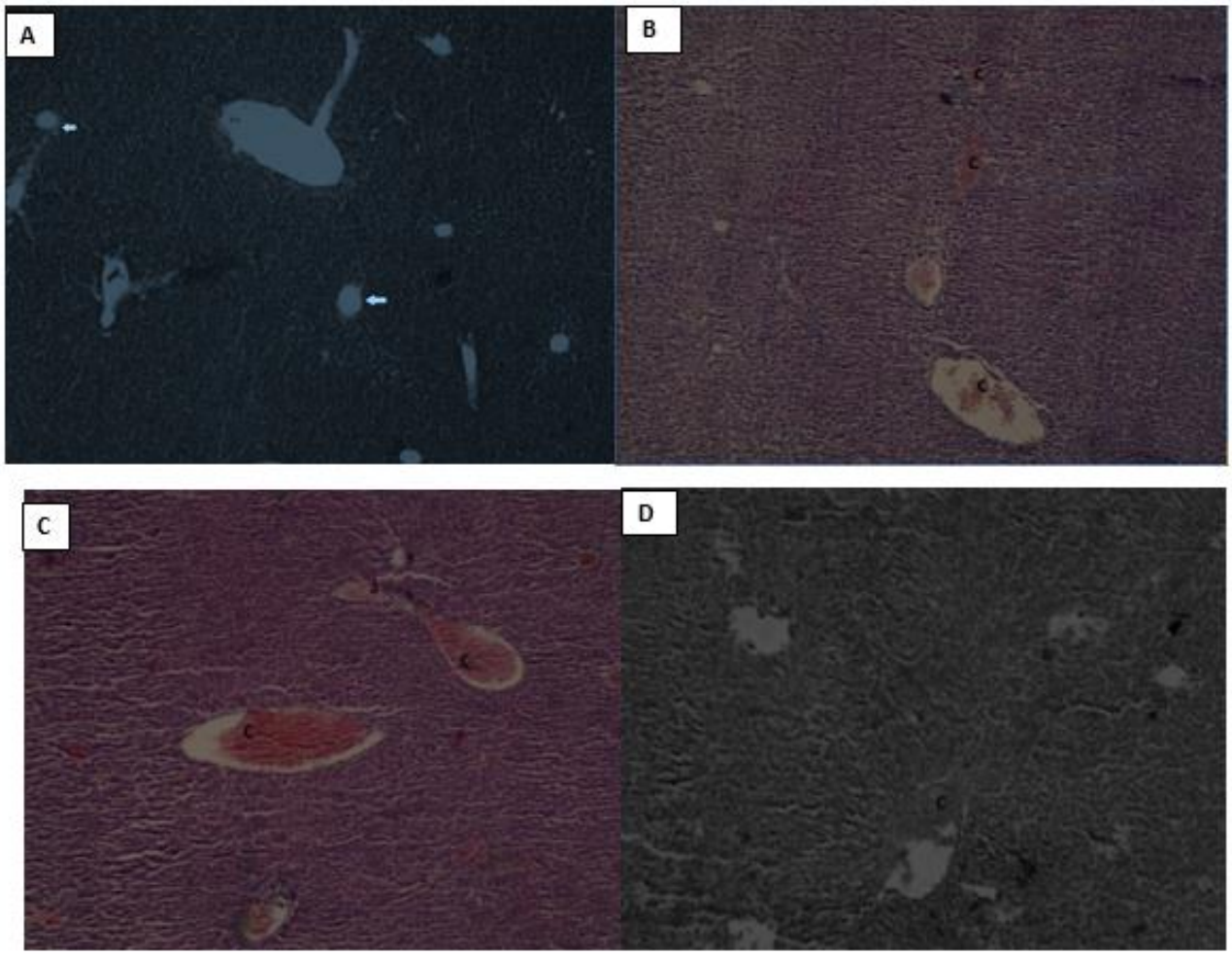

Figure 4.9: Effects of *Croton menyharthii* aqueous root bark extracts in the liver in comparison to the control after a daily administration of different doses for 28 days.

(**A:** Liver section of a control rat that received a daily dose of 1ml distilled water for 28 days (at 100X magnification) (×100)(**PV**-portal vein, **arrow** -central portal vein **B:** Liver section of a rat treated with 200 mg/kg (×100)(**C-** congestion of blood vessels); **C:** Liver section of a rat treated with 400mg/kg Bwt (×100)(**C**-congestion of portal blood vessels; **D:** Liver section of a rat treated with a 800mg/kg Bwt (×400) (**C**-congestion of portal blood vessels)

b) Effects of *Croton menyharthii* DCM/Methanolic root bark extracts in the Liver

Liver tissues from all *Croton menyharthii* DCM/Methanolic root bark extract treatment groups presented with diffuse congestion of portal blood vessels (Figure 4.10).

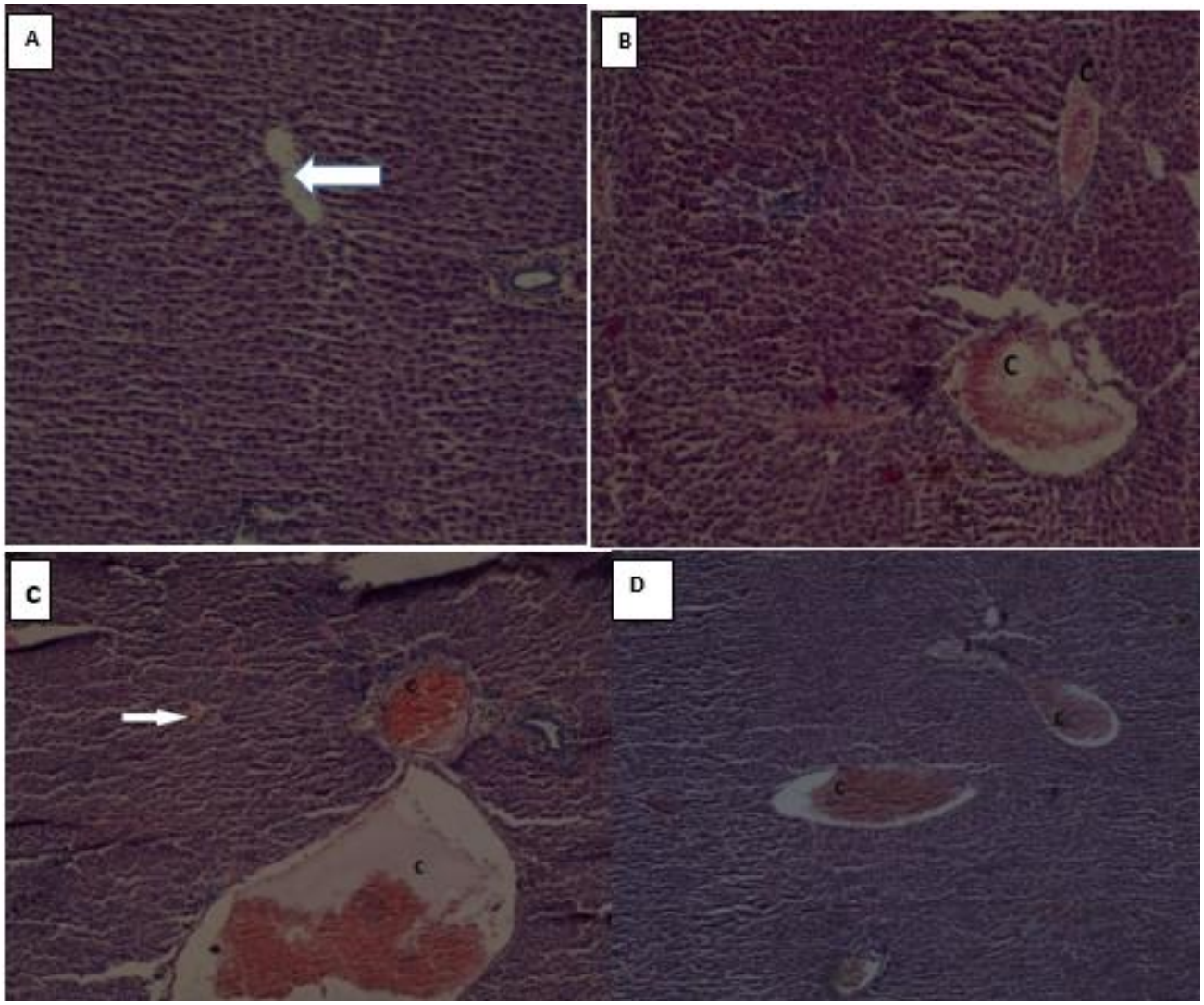

Figure 4.10: Effects of *Croton menyharthii* DCM/Methanolic root bark extract in the liver in comparison to the control after a daily administration of different doses for 28 days.

(**A:** Liver section from a control rat that received a daily dose of 1 ml of extra virgin oil for 28 days (at 100X magnification) (**Arrow**-central portal vein); **B:** Liver section from a rat that received a daily dose 200 mg/kg Bwt(at 100X magnification) (**C**- congestion of portal blood vessels); **C**: Liver section from a rat that received a daily dose 400 mg/kg Bwt (at 100X magnification) **(C-** congestion of portal blood vessels): **D** Liver section from a rat that received a daily dose 400 mg/kg Bwt (at 100X magnification) (**C**-congestion of portal blood vessels).

c) Effects of *Croton menyharthii* aqueous root bark extracts in the kidney

Kidney tissues from rats in all the *Croton menyharthii* aqueous root bark extracts treatment groups presented with congestion of renal parenchymal blood vessels (Figure 4.11).

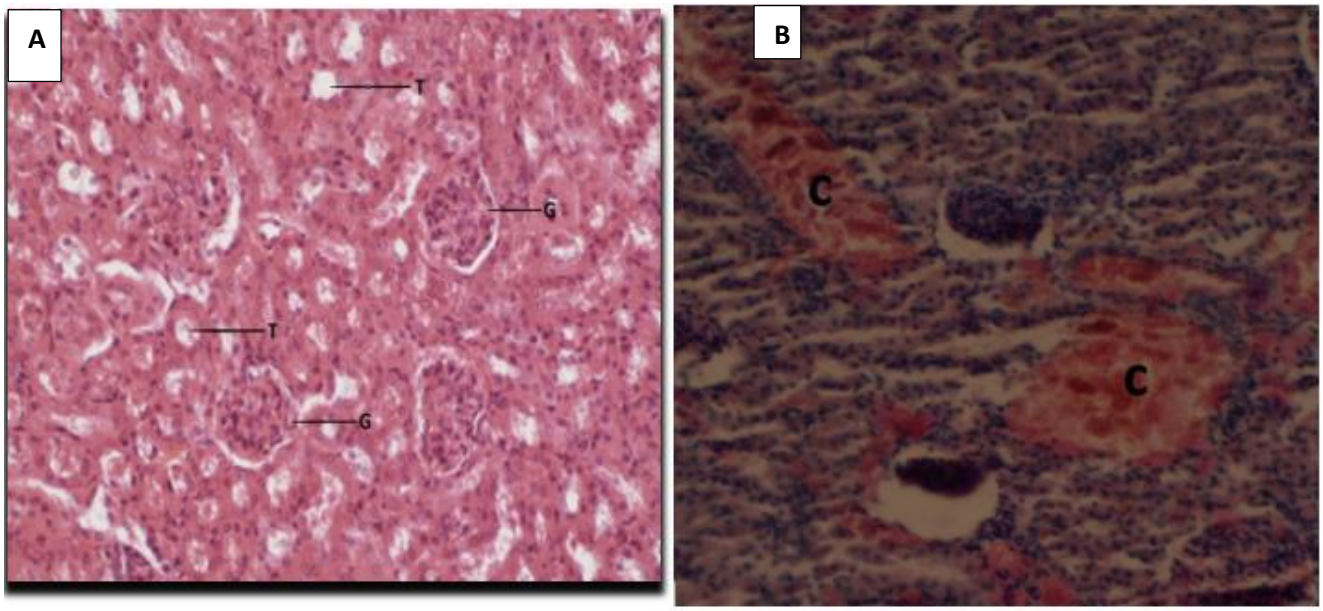

Figure 4.11: Effects of *Croton menyharthii* aqueous root bark extract on the kidneys in comparison to the control after a daily administration of different doses for 28 days.

A: Kidney section from a control rat that received a daily dose of 1 ml distilled water for 28 days (at 400X magnification) (**G**-glomerulus**, T**-renal tubules); **B:** Kidney section from a rat that received a daily dose of 200 mg/kg Bwt for 28 days (at 400X magnification) (**G**-glomerulus, **C**-Congestion of renal parenchymal blood vessels)

d) Effects of *Croton menyharthii* DCM/Methanolic root bark extract in the kidney

There was diffuse congestion of renal parenchymal blood vessels across all Croton menyharthii DCM/Methanolic root bark extract treatment groups. The 200 and 800 mg/kg body weight treatment groups exhibited renal tubular epithelial cell swelling. There was renal parenchyma lymphocytic infiltration in the 200 and 400 mg/kg body weight treatment groups. In addition,

there was multifocal hemorrhage in renal parenchyma in the 400 mg/kg body weight treatment group (Figures 4.12).

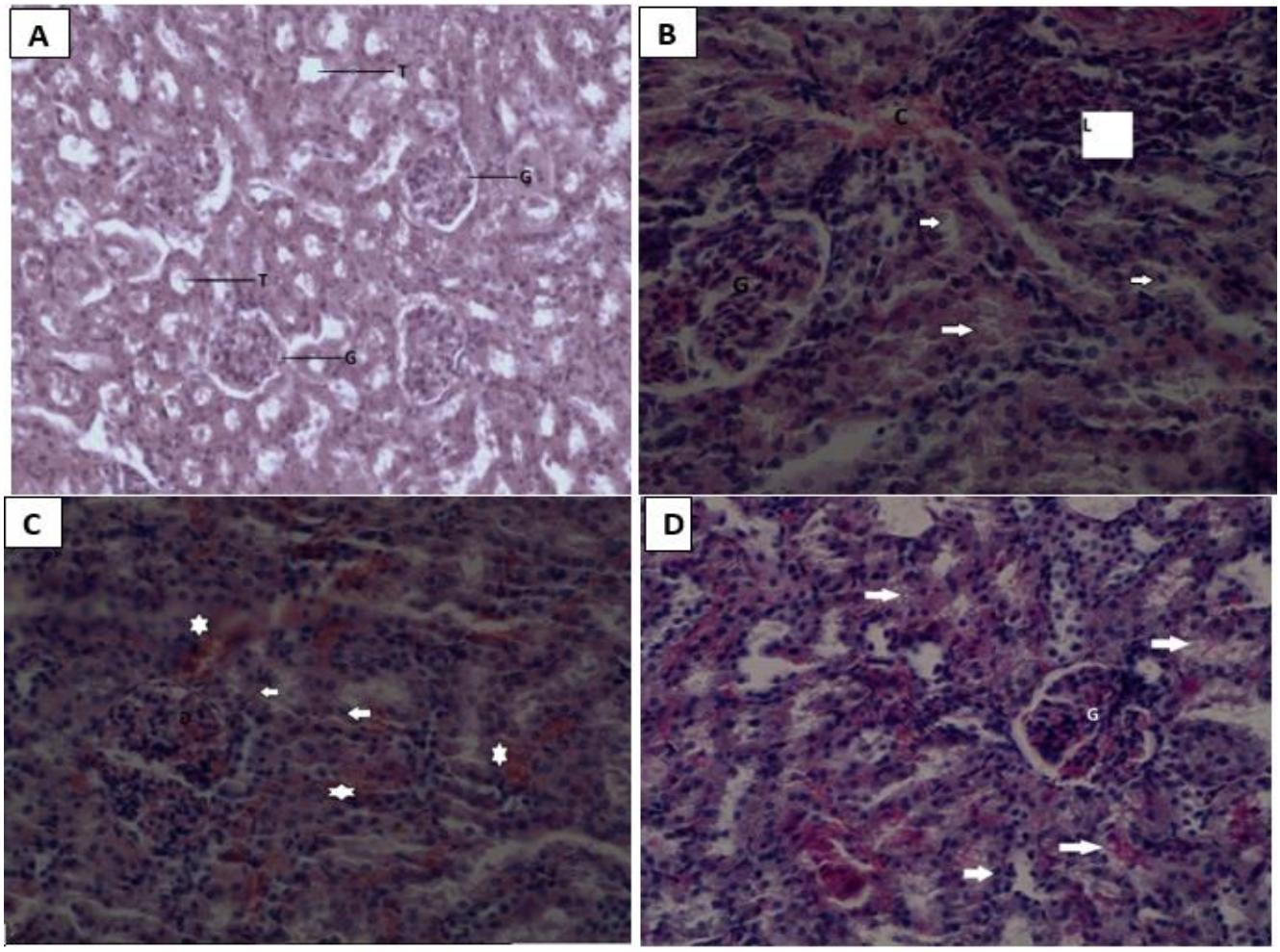

Figure 4.12: Effects of *Croton menyharthii* DCM/methanolic root bark extract on the kidneys in comparison to the control group after administration of different doses for 28 days.

A: Kidney section from a control rat that received a daily dose of 1 ml extra virgin oil for 28 days (at 400X magnification) (**G**-glomerulus**, T**-renal tubules); **B:** Kidney section from a rat that received a daily dose of 200 mg/kg Bwt for 28 days (at 400X magnification) (**G**-glomerulus, **C**-Congestion of renal parenchymal blood vessels, **arrow** - Renal tubular epithelial cell swelling, **L**-Lymphocytic infiltration of renal parenchyma); **C:** Kidney section from a rat that received a daily dose of 400 mg/Kg Bwt for 28 days (400X magnification) (**C**-Congestion of renal parenchymal blood vessels, **Arrow**- focal areas of lymphocytic infiltration of renal parenchyma, **Star-** multifocal hemorrhage in renal parenchyma); **D:** Kidney section from a rat that received a daily dose of 800 mg/Kg Bwt 28 days (at 400X magnification)(**Arrow**-renal tubular swelling, **G**-glomerulus).

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 DISCUSSION

The phytochemical component of a plant determines its pharmacological properties and hence its therapeutic implication (Egamberdieva *et al*., 2017). This is because, phytochemicals possess disease prevention and curative properties and can be used as anti-oxidants, anticancer and antimicrobials among others (Saxena *et al.*, 2013). In this study, the medicinal use of *Croton menyharthii* may be justified by the useful phytochemicals present in the plant.

This study established the presence of alkaloids, saponins, tannins, phenols and cardiac glycosides in both the aqueous and DCM/Methanolic extracts of *C. menyharthii* root barks. Both extracts lacked terpenoids, anthraquinones and flavonoids. In contrast to Aderogba *et al.*, 2013 who reported on the presence of flavonols while using methanolic leaf extracts of *Croton menyharthii*, this study did not establish flavonols in both aqueous and DCM/Methanolic root bark extract of the plant. In addition, most Croton species have diterpenoids as the major phytochemical component (Salatino *et al.*, 2007). However, in this study, both aqueous and DCM/Methanolic root barks extracts lacked terpenoids. This is not unusual since (Barth *et al*., 2018) reported on absence of terpenoids while testing *Croton floribundus* leaves and stem barks.

The use of this plant for fertility control may be justified by presence of some of the phytochemicals in this study. Both aqueous and DCM/Methanolic *Croton menyharthii* root extracts were found to have saponins. Saponins prevent fertilization (antizgyotic), interrupts implantation and also can induce abortion (Kaingu *et al.,* 2017). Alkaloids prevent fertilization, interrupts implantation and have abortifacient activity (Kaingu *et al.*, 2017). They also prevent ovulation, cause resorption of embryos, disrupt the estrus cycle and the endocrine system

(Kaingu *et al.,* 2017). Phenols have been reported to have anti-implantation activity and their presence in this plant could have a synergetic effect on its antifertility effect (Kumar *et al.,* 2017*)*. Tannins have an effect on the endocrine system. They interact with eostrogen receptors, hence their possible role in the anti-fertility activity of this plant (Njeru *et al.,* 2013).

The phytochemical component of the plant can be used to explore other possible uses that are yet to be established. This plant may therefore be a source of novel drugs of pharmaceutical importance (McChesney *et al.,* 2007). In this study the presence of alkaloids was demonstrated. Alkaloids, are a broad group of phytochemicals and have been reported to have antimicrobial activity by boosting white blood cells which fight harmful germs and cell debris in the body (Ogunwenmo *et al*., 2007). They also have analgesic and antineoplastic activity (Njeru *et al.,* 2013). They also affect the central nervous system (CNS) by mimicking some neurotransmitters at the synapses. Some of these are serotonin, dopamine and acetylcholine (Ogunwenmo *et al.,* 2007). This could possibly explain the CNS effects that were observed in this study acute oral toxicity. Alkaloids can cause hallucinations and addiction (Njeru *et al.,* 2013).

In this study, both extracts demonstrated the presence of cardiac glycosides. Cardiac glycosides are pharmacologically useful to the heart. They have positive ionotropic effects by inhibiting the Na+/K+-ATPase pump and can be used in management of congestive heart failure, cardiac arrhythmias and atrial fibrillation. They can also be used as diuretics and emetics (Awoyinka *et al.,* 2007; Harborne, 1998; Ogunwenmo *et al.,* 2007; and Ngoci *et al.,* 2011).

This study also identified phenolic compounds in both extracts. Phenols act as antivirals and are useful in the management of inflammatory conditions and chronic pains (Maobe *et al*., 2012). They also have antioxidant properties hence are useful in prevention of degenerative diseases like inflammation, cancer and cardiovascular diseases (Priya, 2014).

Tannins were present in both extracts. Tannins have free radical scavenging action and hence can act as antioxidants. They also have anti-tumor and antimicrobial activity; anti-fungal, anti-irritant, anti-parasitic, anti-diarrhoeal and anti-septic properties. They therefore can be used to promote wound healing, to arrest bleeding and improve blood vessels health (Awoyinka *et al.,* 2007; Ogunwenmo *et al.,* 2007; Ngoci *et al.,* 2011). Tannins are strong molluscicidals hence can be used in control of snails in a bid to prevent schistosomiasis. Economically, they are useful in the leather industry where they are used to tan leather (Njeru *et al.,* 2013; Saxena *et al.,* 2013). On the negative side, Tannins tend to decrease the digestion of foods especially proteins (Barszcz1 *et al.,* 2011).

Both extracts had saponins. Saponins can be used as a cough remedy as they tend to boost the respiratory system (Barbosa, 2014). They also have anti-protozoal, anti-inflammatory, emetic, molluscicidal, antifungal, antiviral, insecticidal and antibacterial activity (Ngoci *et al.,* 2011; Saxena *et al.,* 2013). Some saponins have been shown to have anticancer properties especially against colon cancer. They also have immunoregulatory activity, anti-oedema, purgative, antitussive, and antithrombotic properties (Njeru *et al.,* 2013). Economically, saponins can be used in the manufacture of detergents and cosmetics (Ngoci *et al.,* 2011). However, saponins tend to reduce the digestion of proteins and vitamins from the digestive tract (Francis et *al.,* 2002).

Acute oral toxicity using both aqueous and DCM/Methanolic extracts of *Croton menyharthii* caused no mortality. The LD50 for both extracts was found to be >2000mg/kg. According to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), *Croton menyharthii* root bark extracts can be classified as category five indicating that, they are of low toxicity (GHS, 2017). According to WHO recommended classification of herbal products, they fall under category 3. This implies that they are slightly toxic (WHO, 2009).

No other study has been done on *Croton menyharthii* but studies on other Croton species have reported similar findings. For example, the LD_{50} for methanolic leaf extract of *Croton zambesicus* in mice was found to be >5000 mg/kg (Onwusonye *et al.,* 2016). Similarly, a toxicological study of aqueous root extracts of *Croton membraneceous* indicated that it had an LD_{50} of >3000 mg/kg (Maroyi, 2018); aqueous leaf extract of *Croton lobatus* was found to have an LD 50 >2000 mg/kg (Lagnika *et al.,* 2016), ethanolic leaf extract of *Croton stellatopilosus* Ohba was found to a have an LD_{50} of 10250 mg/kg (Chaotham *et al.,* 2013) and stem bark extracts of *Croton Macrostachyus* had an LD_{50} >5000 mg/kg body weight (Mbiantcha *et al.,* 2013). Several other studies have been carried out on medicinal plants from the Euphorbiaceae family and declared them safe. Sawadogo *et al*., 2018 reported the LD_{50} of *Jatropha curcasto* to be >5000, Fokam *et al.,* 2017 reported the LD_{50} of *Euphorbia scordifolia* to be >5000 and Njoya *et al.,* 2018 reported the LD_{50} of *Codiaeum variegatum* to be >24000 mg/kg body weight.

However, some plants from the same family have been found to be toxic with an LD_{50} lower than 2000 mg/kg. For example; the LD_{50} of *Oxalis corniculata* and *Phyllanthus fraternus* were found to be 1300 and 1125 mg/kg body weight respectively (Singh & Prakash, 2014), the LD_{50} of *Croton penduliforus* seed oil was reported to be 570 mg/kg (Ojokuku *et al.,* 2015), LD_{50} of *Euphorbia heliscopia* was reported to be 1211.7 mg/kg (Sultan & Hussein, 2006) and that of *Ricinus cummunis* seed was reported to be 1587 mg/kg body weight (Muhammad *et al.,* 2015).

In this study, there were no adverse physical or clinical effects observed in all animals when both extracts were administered. An increase in respiration rate was observed in all treated rats. This is corroborated by Vaghela *et al.,* 2018 who reported similar findings. In addition, all the rats exhibited drowsiness and lethargy which was observed within the first thirty minutes after administration. The rats fully recovered within four hours. These effects are probably due to

the presence of alkaloids that have been shown to have an effect on the CNS (Njeru *et al.*, 2013).

When an extract is orally given via an oil-based vehicle, the resulting mixture is of high viscosity and tend to stick to the sides of the gavaging needle (Turner *et al.*, 2011). This makes the rats' mouths to be in contact with larger amounts of extract in comparison to aqueous based vehicles. This may explain why the treatment groups that received the DCM/Methanolic extract, which was most likely bitter due to presence of alkaloids and tannins (Njeru *et al.*, 2013), excessively rubbed their mouths after dosing. Vaghel *et al.*,2018; Sultan and Hussein, 2006 reported similar findings.

In all treatment groups, there was a gradual increase in body weight compared to the control groups. This indicates that the extract had no inhibitive effect on the growth of the animals and did not affect the metabolism of fats, proteins and carbohydrate (Klaassen, 2001). At 5000 mg/kg bodyweight of the aqueous extract, the weight increase was significantly lower. This may indicate that, at high doses, the aqueous extract had a slight inhibitory effect on the growth of rats as a result of alteration in fats, proteins or carbohydrates metabolism. This is similar to what has been reported by Roy *et al.,* 2016 and Taziebou *et al.,* 2007.

The rats that received the organic extracts had a higher percentage weight increase than those that received aqueous extract. This may be as a result of using oil-based vehicles during gavage which leads to high stress levels resulting in the activation of the hypothalamus-pituitary-adrenal pathway. This might have led to increased levels of corticosterone hormone which has been associated with an increase in weight gain (Márquez *et al.*, 2002; Brown *et al*., 2000).

Daily administration of *C. menyharthii* aqueous and DCM/Methanolic extracts did not have adverse physical and clinical effects on the rats in all the treatment groups over the 28 days. The findings are similar to those observed in rats treated with *Croton membranous* and *Croton*

zambesicus (Maroyi, 2018; Onwusonye *et al*., 2016). The rats that received the organic extract were seen to excessively rub their mouths on the walls and the floor of their cages. They also scrambled for water immediately after dosing. These were signs of irritation which could have been as a result of the presence of tannins and alkaloids which are bitter (Njeru *et al.*, 2013) and the fact that the oil based extract was sticky and adhered to the rats' mouth during gavage (Turner *et al.,* 2011). These findings correlate to those of Vaghel *et al.*, 2018 and; Magili and Bwatanglang, 2018.

Determination of food and water consumption is one of the parameters that is used to determine whether a substance is toxic or non-toxic (OECD 407, 2008). Significant changes in feed and water consumption observed among the treatment groups were not dose related. This might therefore be due to intergroup and/or intra-animal related factors and not due to the extract administration. Water consumption among the rats that received the DCM/Methanolic extract was non-significant compared to the control. Water consumption was significantly higher at lower aqueous extract treatment groups (200 and 400 mg/kg) compared to the control (Table 6). The rats that received the DCM/Methanolic extract consumed less feeds and water compared to those that received the aqueous extract. This can be attributed to extra virgin oil's tendency of delaying gastric emptying and reducing appetite (Damgaard *et al.*, 2013).

Weekly mean weight changes are used to determine the toxicity of a substance in sub-acute toxicity testing (OECD 407, 2008). Both extracts caused a non-significant ($P > 0.05$) higher increase in mean weight gain in all treatment groups compared to the controls. The weights of all rats increased consistently throughout the study period. Therefore, both extracts had little effects on the rate of growth. Probably; fat, protein and carbohydrates metabolism was not disturbed (Klaassen, 2001). These findings correlate with Adesina *et al.*, 2019 who reported non-significant changes on rats' body weights by *Phyllanthus fraternus* Schum. and Thonn (Euphorbiaceae) after 28-days oral administration.

The laboratory assessment of haematological parameters in sub-acute toxicity study is vital in determining the toxic effect of a substance. When a substance is bioavailable at toxic levels in biological media, then it can induce changes in the various blood parameters. This might be due to its possible toxic effects in the spleen or in the bone marrow causing possible alteration in haematopoiesis and or through production of antibodies against the blood-forming precursors or peripheral blood cells destruction (Arika *et al.,* 2016).

In this study, there was no significant change observed in the haematological parameters by both the aqueous and DCM/Methanolic extract. This in an indication that the extracts may not have a major effect on the bone marrow or the spleen. Probably the extract did not cause peripheral blood cells destruction or stimulate antibodies against blood forming precursors. These findings correlate with those of Sharif *et al*., 2015 who reported non-significant effects on haematological parameters in rats by *Euphorbia pulcherrima* (Euphorbiaceae).

The aqueous extract caused a non-significant dose dependent increase in WBC counts and a non-dose dependent increase in the platelets compared to the control. Probably the extracts had a positive effect on hematopoietic growth factors that activate hematopoiesis. These are thrombopoietin and cytokines (interleukins and colony stimulating factors) which increases platelet and WBC precursors respectively. This may be explored in use of the plant as an immune booster, for haemophilia and other bleeding disorders and/or in wound healing (Arika *et al.,* 2016). These findings are contrary to what Okonkwo *et al.,* 2019 reported. Insecticidal oils from *Euphorbia milii* (Euphorbiaceae) caused a reduction in platelets and WBC in rats.

A non-significant non-dose dependent rise in platelets levels was observed in the rats that received the DCM/Methanolic extract. The extract possibly had a non-significant stimulatory effect on thrombopoietin. This was contrary to what Sawadogo *et al.,* 2018 reported. *Jatropha curcas* L (Euphorbiaceae) caused a decrease in platelets levels in rats after 28-days oral

administration. The DCM/Methanolic extract also resulted in a non-significant dose dependent rise in RBCs and HB and a dose dependent non-significant fall in MCV and MCH. This could mean that the extract has a negligible tendency of inducing microcytic anaemia. It is possible that, the extract negligibly affected iron absorption which could result in iron deficiency on long term administration (Urrechag *et al.*, 2014). These findings corroborate those of Okonkwo *et al*., 2019 who reported a fall in MCH and MVC in rats, but at the same time contrasts them, as he reported a reduction in RBC, upon a 28-day administration of insecticidal oils from *Euphorbia milii.*

The liver and the kidneys play a major role in metabolism of substances that enter the body. Both have microsomal cytochrome P450 monooxygenases (CYP450) that are vital in the metabolism of several chemical compounds. The majority are in the liver but the kidneys also has them in the renal proximal tubule making it a target for chemical-induced nephrotoxicity. The liver is susceptible to toxicity of oral administered substances. This is because majority of substances are transported to the liver for biotransformation which might give rise to more toxic compounds.

The nephrotoxic compounds, apart from being produced by the kidneys may also be transported to the kidneys from other parts of the body for excretion. This makes the kidneys more susceptible to chemical-induced nephrotoxicity (Suter *et al*., 2004; Van *et al*., 2003; Pizzo *et al*., 2015). Therefore, this formed the basis of evaluation of liver and kidney function tests in this study.

Tissues in the body tend to release certain enzymes into the circulatory system upon injury. Alanine amino transferase (ALT) and Aspartate amino transaminase (AST), are enzymes present in liver cells. If a compound causes some injury to the liver hepatocytes, they release these two enzymes. The two are then detected in serum to show the extent of injury (Ike *et al.*,

2016). ALT is more specific to liver injury since AST is also present in other organs like the heart and muscles (McGill, 2016). The liver plays a big role in protein synthesis (e.g. albumin) in the body that is vital in fighting infections. A reduction in albumin and total protein levels in the serum may indicate liver injury, liver disease or protein deficiency due to malabsorption (Thapa and Walia, 2007).

In this study, the aqueous extract caused a dose related significant decrease in protein levels compared to the control. This correlates to Gadir *et al.,* 2003; who reported the reduction of total proteins in goats by *Croton macrostachys*. Djimeli *et al.,* 2017 also reported a reduction of serum protein levels in mice by *Alchornea cordifolia* (Euphorbiaceae). Adesina *et al.,* 2019 also reported a significant reduction in protein levels by *P. fraternus* leaf extracts in rats.

However, the extract caused a non-significant effect on ALT and AST levels. The extract therefore, most probably caused a reduction in protein levels due to malabsorption in the gut. This could be due to tannins and saponins which were present in the plants extract. Usman *et al.,* 2013 reported similar findings on his study on *Euphorbia lateriflora* in rats.

The DCM/Methanolic extract caused a non-significant effect on ALT, AST and protein levels. However, the non-significant reduction of protein levels indicated a possibility of protein malabsorption within the rat gut. These findings are similar to those reported by Agbaje *et al.,* 2009. The findings however are in contrast to those reported by Adedapo *et al*., 2004 who illustrated that five plants from the genus Euphorbia caused a significant increase in serum total protein, ALT and AST levels in rats.

In subacute toxicity testing, kidney function tests are vital since they aid in finding out whether the test substance has a potential to cause renal damage. Serum creatinine levels, an indicator of glomerular filtration rate is a major biomarker of renal function. Other important tests done to evaluate the potential of kidney damage by substances are BUN and urinalysis. Creatinine

and urea are end products of protein metabolism. An alteration in glomerular filtration due to kidney damage can result to their build-up in the body (Krstic *et al.,* 2016).

In this study, serum creatinine and BUN levels were evaluated in all the rats. Both aqueous and DCM/Methanolic extract caused non-significant alterations of serum creatinine and BUN levels. This indicates that both extracts did not cause physiological injury to the kidneys. However, there was a dose dependent increase in the levels of creatinine in the treated groups. These findings are similar to those of Adesina *et al.,* 2019. The non-significant low urea levels in all treatment groups for both extracts, can be explained by the low protein levels. This is because, urea is a product of protein metabolism (Higgins, 2016) whose levels were significantly low in all aqueous extract treatment groups, and non-significantly low in the DCM/Methanolic extract treatment groups. These findings correlate to those reported by Ikewuchi *et al.,* 2011 and Usman *et al.,* 2013.

Macroscopic examination showed no change in colour, size or texture of the heart, lungs, liver, stomach, kidneys and the spleen in all treatment groups. This may be an indication that both aqueous and DCM/Methanolic extracts did not cause macroscopic pathological changes in the heart, lungs, liver, stomach, kidneys and the spleen. Similar findings have been reported by Lagnika *et al.,* 2016, Chaotham *et al.,* 2013 and Njoya *et al.,* 2018.

The liver and the kidneys relative-organ weight ratios were determined since they are the major metabolic and excretory organs respectively. At 200 mg/kg body weight DCM/ Methanolic extract, there was a significant decrease in the mean relative-organ weight ratio of the liver. This was not dose dependent and might be due to inter-group and/or intra-animal biological variations. All the other extract doses did not cause a significant effect on the relative-organ weight ratios of the liver and kidneys. These findings are in contrast to those of Chaotham *et*

al., 2013 who reported a dose related significant increase in relative organ weight of the liver and kidneys in rats treated with *Croton stellatopilosus* extract.

The histopathological evaluation of the liver and the kidneys did not show any marked findings in rats that received the aqueous extract. This further confirms that the low protein levels that were observed in all the aqueous treatment groups were probably as a result of protein malabsorption and not liver or kidney damage.

The histopathological findings for the DCM/Methanolic extract did not show any major signs of toxicity in the liver. There was mild congestion of portal blood vessels in all the treatment groups when compared the control group. This is contrast to the findings by Akinloye *et al.,* 2015 who reported liver injury and necrosis in rats treated with *Croton zambesicus* extract. The findings are also contrary to Rodrĺguez *et al.,* 2004 who reported nuclear alterations, microvacuolar degeneration and turbid tumefaction in the liver in rats treated with *Croton cajucara* extract.

Kidney histopathological evaluation showed that there was probable kidney damage in all the DCM/Methanolic extract treatment groups. There was renal tubular epithelia cell inflammation lymphocytic infiltration and multifocal haemorrhage within renal parenchyma of the treatment groups. This was an indication that the DCM/Methanolic extract might have a lethal effect on the kidneys resulting in renal tubular damage. Makoshi *et al.,* 2016 reported similar findings. *Acalypha wilkesiana* (Euphorbiaceae), caused severe necrosis and degeneration of the tubules and glomeruli in rats after 14-days oral administration. However, the plant also caused hepatotoxicity which was not observed in this study.

Biochemical parameters did not correlate with the kidney histopathological findings. There was non-significant difference compared to the controls. This is because, by the time a rise in serum creatinine levels is detected, the kidney function would have reduced by 50%. Serum

creatinine which is more precise in evaluation of renal function than urea is therefore a late measure of renal injury (Gounden and Jialal, 2019). Therefore, the histopathological changes observed in this study were probably an indicator of early signs of kidney damage due to DCM/Methanolic *C. menyharthii* root bark extract administration.

The histopathological changes were not observed in the groups treated with the aqueous extract. This is possibly because, oil-based vehicles, in this case, extra virgin oil, tend to increase the rate and extent of absorption of both fat-soluble and sparingly water-soluble substances from the gut. This may result in the substances being conveyed to the site of action at a faster rate and in enormous amount causing an increased likelihood of toxicity than aqueous-based vehicle (Marty *et al.*, 2007).

5.2 Conclusions

1. *Croton menyharthii* root barks have vital phytochemicals such as alkaloids, saponins, tannins, phenols and cardiac glycosides that may be responsible for its traditional use as an antifertility agent and other mentioned medicinal uses. The phytochemicals place a bed of exploration and research of more possible uses of this plant.
2. Single doses of C. *menyharthii* that are less than 2000 mg/kg body weight may be considered safe.
3. Oral administration of the *C. menyharthii* decoction may have an effect on protein digestion on long term use. *Croton menyharthii* is a possible nephrotoxicant. Long term administration of the extract could lead to detrimental effects in the kidneys as was evident in the histopathological findings.

5.3 Recommendations

1. Quantitative analysis of *C. menyharthii* plant phytochemical component needs to be done.
2. Sub chronic and chronic studies of *C. Menyharthii* need to be done in order to have a clearer picture of the toxicity profile of the plant in terms of nephrotoxicity, hepatotoxicity, cytotoxicity and mutagenicity. This is because, the plant is used for fertility control which is long term.
3. Other solvents/solvents mixtures extraction need to be carried out in order to have a better picture of the best solvent to have maximal extract yield. The consequent solvent extract toxicity profiles should also be performed.
4. A study need to be done in order to determine the actual dosages used by herbalists
5. Pharmacokinetic studies need to be done in order to determine the therapeutic dose and dose frequency. This in turn will help forecast whether the dose will be well tolerated

6. People need to be advised on potential harm of *C. menyharthii* decoction when taking it together with food.
7. Due to *Croton menyharthii* nephrotoxicity potential on long term use, people with renal function anomalies should be cautioned against its use. Those who use it should have their renal function monitored closely.
8. A detailed study on the effects of *Croton menyharthii* on weight gain, digestion, food and water intake need to be done.

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Tana River County first county integrated development plan (CIDP) J**uly 2013 – June 2018.** Page 41

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APPENDICES

Ethical Approval Form

UNIVERSITY OF NAIROBI
FACULTY OF VETERINARY MEDICINE
DEPARTMENT OF VETERINARY ANATOMY AND PHYSIOLOGY

P.O. Box 30197,
00100 Nairobi,
Kenya.

Tel: 4449004/4442014/ 6
Ext. 2300
Direct Line. 4448648

Ms. Emma Wanjiku Kagunya
Department of PHP&T

REF: FVM BAUEC/2019/185

25th January 2019

Dear Ms. Kagunya

RE: Approval of Proposal by Biosafety, Animal use and Ethics committee

Phytochemical and toxicological evaluation of *Croton menyharthii* from Tana River County, Kenya.

By Emma Kagunya (J56/7684/2017)

We refer to your revised MSc proposal submitted to our committee for review and your application letter dated 24th January 2019.

We have reviewed your MSc. proposal, particularly section 3.4 and 3.5 that involves use of laboratory rats for acute and subacute toxicity tests. We are satisfied that the proposed treatment and care of the animals meets acceptable standards for animal welfare. Furthermore, the numbers proposed are reasonable.

We have also noted that Prof James Mbaria will supervise the animal experiments and humane euthanasia.

We hereby give approval for you to proceed with the experiments as outlined in the submitted proposal.

Yours sincerely

Kaluwa

Dr. Catherine Kaluwa, BVM, MSc, Ph.D
Chairperson,
Biosafety, Animal Use and Ethics Committee
Faculty of Veterinary Medicine.