SNAKE-ANTIVENOM ACTIVITY OF SELECTED MEDICINAL PLANT EXTRACTS FROM TURKANA AND UASIN-GISHU COUNTIES OF KENYA

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DECLARATION

I, Yego Kennedy Kimurgor, duly declare that this thesis is my original work and has not been presented for a degree in any other university or for any other award

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DEDICATION

To Noah Kiptarus Arap Rutto from whom I derive my motivation; rest in peace grandpa

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ABBREVIATIONS AND ACRONYMS

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AQAS	Aqueous extracts of *Amaranthus spinosus*
AQCA	Aqueous extracts of *Cyperus articulatus*
AQCS	Aqueous extracts of *Carissa spinarum*
AQCM	Aqueous extracts of *Combretum molle*
AQRU	Aqueous extracts of *Rhynchosia usambarensis*
AST	Aspartate aminotransferase
BUN	Serum urea nitrogen
CREAT	Serum creatinine
D-BIL	Direct bilirubin
ED_{50}	Median effective dose
EDTA	Ethylenediaminetetraacetic acid
GGT	Gamma glutamyl transferase
Hb	Hemoglobin concentration
I.p	Intraperitoneal
I.v	Intravenous
LD_{50}	Median lethal dose
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
PCV	Packed cell volume

PLT	Platelets
RBC	Red blood cells
T-BIL	Total bilirubin

ABSTRACT

The present management regime of snake bites requires the use of antivenom immunoglobulins. However, these antivenoms have the limitations of being expensive, requiring cold storage facilities and have problems of hypersensitivity reactions in some individuals. *A. spinosus, C. articulatus, C. spinarum, C. molle* and *R. usambarensis* have been used traditionally in the management of snake bites in Turkana and Uasin-Gishu Counties, Kenya. However, their efficacy and safety have not been scientifically validated. The aim of this study was therefore to determine *in vivo* and *in vitro* efficacy and safety of these selected medicinal plants using the mouse model, agarose-erythrocyte-egg yolk gel plate and human citrated plasma methods. Relevant plant parts from these medicinal plants were collected, dried under shade, crushed into powder and then extracted with distilled water. The potency of the antivenom activity of the plant extracts was estimated by determining the least dose of venom required to kill 50% of the mice (LD_{50}) and the dose of the extract required to protect half the animals (ED_{50}) in a statistically significant group of animals from two times the LD_{50}. The antivenom studies suggest that the aqueous plant extracts possess antivenom activity against *Naja subfulva* venom both *in vivo* and *in vitro*. Evaluation of acute and sub-acute toxicity studies indicated no lethality after intraperitoneal administration of the extracts in mice at 1600, 2500 and 5000 mg/kg body weight. Repeated daily oral administration (sub-acute studies) of the five aqueous plants extracts at 10, 300 and 1000 mg/kg body weight to mice for 14 days demonstrated significant decreases in the average weekly body weight, increases in percent organ to body weight, decreases in several biochemical analytes and enzymes, and increases in white blood cell and differential white blood cell count. However, no changes in the level of red blood cells, hemoglobin and the related indices were observed except for *C. spinarum* extract-treated mice where red blood cells, hemoglobin and packed cell volume were decreased. Phytochemical screening of the five aqueous plants extracts demonstrated the presence of phenols, alkaloids, flavonoids, flavones, steroids, terpenoids, triterpenoids, tannins, saponins and cardiac glycosides. Of the seventeen mineral elements levels estimated in the five aqueous plants extracts, twelve were below the recommended daily allowances and five were above the recommended daily allowances. In conclusion, the aqueous extracts of *A. spinosus, C. articulatus, C. spinarum, C. molle* and *R. usambarensis*, neutralized snake venom activity of *Naja subfulva* and demonstrated toxicity in the subacute toxicity studies. The observed antivenom activity and subacute toxicity could be explained by the phytochemicals and mineral elements present in these five aqueous plants extracts from Turkana and Uasin-Gishu counties. The five studied plants could therefore be used at the tested extract efficacy doses.

CHAPTER ONE

INTRODUCTION

1.1 Background

Snake envenomation is a serious medical problem in many parts of the world, especially in tropical countries. Globally, the incidence of clinically significant snake bite envenoming has been estimated at 2.5 million and out of these about 94,000-125,000 are fatal (Kasturiratne *et al*., 2008; Ramos and Ho). In Kenya, a preliminary survey based on Ministry of Health and medical facilities records in Kakamega, Lake Baringo, Laikipia, Kilifi and Malindi and northern Kenya suggested an overall average frequency of snakebite of 14 (range 2-68) per 100 000 population per year (WHO, 2010a). Moreover, a community-based study in Kilifi reported 15 adult snakebite fatalities per 100 000 population per year (Snow *et al*., 1994). The recommended method of treating snake bite envenomation effectively is through antivenoms; a serum separated from blood of hyper immunized horses. The first snake antivenoms immunoglobulin, called anti-ophidic serum, was developed by Albert Calmette in 1895 against envenomation by the Indian cobra, *Naja naja* (Gomes *et al*., 2010).

World Health Organization has designated the Liverpool School of Tropical Medicine as the International Collaborating Centre for anti-venom production and testing (Theakston *et al*., 2003). Sub Saharan African antivenom manufacturers are; South African Vaccine Producers, MicroPharm, VINS Bio, Instituto Bioclon, Bharat Serums and Vaccines, Instituto Butantan and Instituto Clodomiro Picado (Brown, 2012). Antivenoms neutralize the effects of snake venom thereby stopping further damage to the body, but it does not, however, reverse the already done damage. Other limitations of snake antivenom therapy include, its unavailability, high cost, and

inadequate storage facilities in developing countries as well as difficulty in identifying the snakes. Some individuals may also suffer from an immediate hypersensitivity reaction after antivenoms treatment (Cannon *et al*., 2008).

Several plants are known to have snake antivenom activities but their efficacy is often unproven (Chatterjee *et al*., 2006). World Health Organization (WHO) in 2002, however, launched a global plan to make the use of traditional medicine safer by encouraging evidence-based research on their efficacy, safety and quality (Tanaka *et al*., 2009).

1.2 Statement of the Problem and Justification

Animal-derived antivenom constitutes the only validated therapy for snakebite envenoming (WHO, 2007a). However, the current crisis in anti-venom supply to Sub-Saharan Africa has prompted a need to search for alternative snake venom inhibitors, either synthetic or natural, which would substitute or complement the animal derived anti-venoms. The antivenom supply crisis is attributed to high costs of some available products, lack of commercial incentives for manufacturers, ignorance of true anti-venom requirements, deficient purchase systems, loss of confidence of antivenom therapeutic efficacy and safety (Theakston, 2002; Cheng and Winkel, 2004).

Herbal medicines for snakebite treatment are readily available in rural areas. However, while scientific studies have validated some traditional remedies by confirming their biological activity (Fiot *et al*., 2006; Palombo, 2006), most phytochemicals are being used in various parts of the world as antidotes to snake envenomation but their inhibitory action is seldom studied (Perumal

Samy *et al*., 2012). Therefore, their use still remains contentious due to doubts about their efficacy and safety. It is for this reason therefore, that this study's aim was to determine *in vivo* and *in vitro,* using the mouse model, the efficacy and safety of five selected Kenyan medicinal plants used to treat snake bites in parts of Uasin-Gishu and Turkana counties. Plant extracts' efficacy and safety studies will validate or invalidate the plants under study for continued use or otherwise. Validated herbal remedies will act as a blue print for new drugs and consequently enhance conservation of the medicinal plants.

1.3 Null hypotheses (H_O)

i) The five aqueous plants extracts used in the management of snakebites are not effective *in vivo* and *in vitro* as snake antivenoms.

ii) The five aqueous plants extracts used in the management of snakebites are unsafe.

iii) Phytochemicals and mineral elements present in the five aqueous plants extracts do not possess antivenom activities and are unsafe.

1.4 Objectives

1.4.1 Main objective

To investigate *in vitro* and *in vivo* antisnake venom activities and safety of the five aqueous plants extracts from Turkana and Uasin-Gishu Counties against *Naja subfulva* snake venom.

1.4.2 Specific objectives

(i) To determine *in vivo* and *in vitro* efficacy of aqueous extracts of the five selected medicinal plants extracts against *Naja subfulva* snake venom.

(ii) To examine the *in vivo* acute and subacute toxicity of the five aqueous plants extracts used in the management of snakebites in the mouse model.

iii) To explore the phytochemical and mineral elements composition of the five aqueous plants extracts traditionally used as snake antivenoms.

CHAPTER TWO

LITRATURE REVIEW

2.1 Biology of snakes

2.1.1 Description

Snakes are long bodied scaly reptiles with no limbs. More than 2000 snake species have venom toxins (Fry *et al*., 2006) and out of these, less than 200 species have caused clinically severe envenoming in humans, ending in death or permanent disability (Brown, 2012). Distinctive features and characteristics of venomous snakes as described by Otten (1987) include: Triangular shaped head, presence of second set of pits below the nostril of a snake and the presence of a rattle.

2.1.2 Classification

Snakes are mainly classified according to family and fang orientation (Vitt and Caldwell, 2008). Common venomous snake families include: atractaspididae such as burrowing asp (Plate 2.1), elapidae like cobras (Plate 2.2) and mambas, viperidae such as rattle snakes (Plate 2.3) and puff adders, hydrophildae and colubridae like boomslangs (Plate 2.4). There are three types of venomous snakes described on the basis of fang orientation: opisthoglyphs-rear-fanged snakes, proteoglyphs-fixed front fang snakes and solenoglyphs which have movable front fangs (Bauchot, 2006).

Plate 2.1: *Atractaspis engaddensis* (Source: Wikipedia)

Plate 2.2: *Naja subfulva* (Source: www.africanreptiles-venom.co.za)

Plate 2.3: *Crotalus atrox* (Source: Robert Roy Britt)

Plate 2.4: *Dispholidus typus* (Source: Paul Donovan)

2.2 Scientific classification and description of *Naja subfulva*

Naja subfulva belongs to the family *Elapidae,* genus *Naja* and species *Naja subfulva*. Its binomial nomenclature is *Naja subfulva* and its common name is *Forest cobra*. *Naja subfulva* is among the largest cobra of the genus *Naja*. The largest recorded being specimen JPT1856 in Zinave, Mozambique whose length was 2396 mm, its tail truncated (Wuster *et al*., 2018). Most adult populations have a brown forebody with spots that are generally black or darker posteriorly. It may have 2 or 3 faint yellow divided crossbars on the neck (Wuster *et al*., 2018). It has 19 mid-body rows of scales; those found along the Kenyan and Tanzanian coast regions are exceptional because they have 17 rows of scales.

2.2.1 Behaviour and diet

It is quite aggressive when provoked hissing loudly while raising its fore body off the ground and spreading its hood. It can strike quickly at long distance and will also rush forward in an effort to strike. It consumes a wide variety of prey which includes, amphibians, lizards, rodents, eggs, fish, birds and other smaller animals and snakes.

2.2.2 Ecology, habitat and distribution

It is well adapted to many environments. It is largely terrestrial but can also climb trees and can swim well in water too. It is mainly diurnal in uninhabited areas but can be nocturnal in heavily populated areas usually found in piles of junk or unused buildings. It usually takes cover in holes, hollow logs, and rock crevices or in termite hills when inactive. It inhabits forests, woodland, coastal thickets, moist savanna and grasslands, from sea level to 2500 m.

2.3 Snake venom

Venoms are toxic saliva consisting of mixtures of bioactive molecules that comprise proteins, inorganic ions and nucleotides. However, proteins constitutes about 90% of the venom and can be categorised as enzymatic proteins or non-enzymatic toxins (Kasturiratne *et al*., 2008). Snake venom has more than 20 types of toxic enzymes and out of these, 12 are found in all types of snake venoms (Koh *et al*., 2006). They include phospholipase A_2, L-Amino acid oxidase, phosphodiesterase, 5'-Nucleotidase, phosphomonoesterase, eoxyribonuclease, ribonuclease, denosinetriphosphatase, hyalirodinase, NAD-Nucleosidase, arylamidase and peptidase (Kang *et al*., 2011). The major venom components that have grave clinical effects are myolitic phospholipases A_2, haemolytic pre-and post-synaptic neurotoxins, cytolytic or necrotic toxins, procoagulant enzymes, and haemorragins (WHO, 2007a).

Calcium, zinc and magnesium divalent metals are also found in venoms of all snake species (Friederich and Tu, 1971). Mineral elements such as iron, copper, and manganese are also present in venoms of most snake species while sodium and potassium monovalent cations are found in high concentrations in all venoms. The mineral elements present in venoms are thought to act as charge balancing ions for inorganic salts and the proteins in venoms; however, venom lethality is not affected by removal of the trace metals from venom but changes its degree of haemorrhagic activity (Koh *et al*., 2006). The haemorrhagic activity disappears when the divalent mineral elements are removed from snake venoms. Moreover, venom's proteolytic activity is significantly decreased in EDTA treated venom (Bottrall *et al*., 2010). It has also been noted that high concentrations of zinc or magnesium restores considerable amount of proteolytic and haemorrhagic activities (Fry *et al*., 2006).

Plate 2.5: snake venom (Source: Atul Kaushika *et al*., 2013)

2.4 Classification of clinical patterns of snakebite envenoming

There are four main types of envenoming namely: cytotoxic, haemorrhagic, neurotoxic and myotoxic envenoming.

2.4.1 Cytotoxic envenoming

This is characterised by blood stained tissue fluid coming from the bitten area, severe pain throughout the affected limb, possible irreversible death of tissue as well as hypovolemic shock.

2.4.2 Haemorrhagic envenoming

This is characterised by bleeding of recent and partly healed wounds, gums, gastrointestinal tracts as well as the genitourinary tracts.

2.4.3 Neurotoxic envenoming

It is characterised by ptosis, paralysis of eye movements, moderate or absent local swelling, painful and enlarged lymph glands draining the site, saliva may become profuse and stringy and difficulties in swallowing and breathing may eventually occur.

2.4.4 Myotoxic envenoming

It is characterised by small local swelling, myalgia, and a progressive descending paralysis resulting in difficulty in breathing (Warrell, 2010).

2.5 Antivenoms

Animal-derived antivenoms constitute the only validated therapy for snakebite envenoming (WHO, 2010a). Snake venom antiserum is of equine origin obtained from the plasma of horses, donkeys, mules, and sheep that are hyperimmunised against the venoms of common venomous snakes (Sarkhel, 2011). Polyspecific antivenoms are obtained from animals hyperimmunised against the venoms of many snake species, while monospecific antivenoms are derived from animals immunised against the venom of a single snake species. Although antivenoms have viable antidotes, they do not provide enough protection against venom induced nephrotoxicity and haemorrhage necrosis (Cheng and Winkel, 2004). However, clinical observations have suggested that antivenoms may prevent local necrosis after bites by *Naja mossambica, Bitis*

arietans and *Naja nigrocollis* if adequate doses are administered within three to six hours of envenoming (Stock *et al*., 2007).

2.6 Herbal antidotes against snakebites

Plant use against the effects of snakebite envenomation was documented by Henri Bocquillon - Limousin (1891). He scientifically screened a number of plant constituents used to treat snake bites but failed, however, to report their efficacies against snake envenomation (Gomes *et al*., 2010). In the past 20 years, however, scientific screening of plants used to treat snake envenomation around the world has been given more scientific attention (Bahekar and Kale, 2013).

Some 600 species of plants from about 100 families are used around the world, either singly or in combination against snake envenomation and are reputed to neutralize the action of snake venom (Perumal *et al*., 2012). A number of Indian medicinal plants are recommended as snake bite antidotes (Bahekar and Kale, 2013); for instance, methanolic root extracts of *Emblica officinalis* and *Vitex negundo* when screened for antisnake venom activity significantly neutralised *Naja kaouthia* and *Vipera russellii* snake venom. Moreover, *V. Russellii*'s coagulant, haemorrhagic, inflammatory and anticoagulant activities were significantly neutralised by extracts from the two plants (Alam and Gomes, 2003). Butanolic extracts of *Eclipta prostrate* plant partially inhibited hemorrhagic activity of most snake venom (Pithayanukul *et al*., 2004). Lupeol acetate compound isolated from the root extracts of *Hemidesmus indicus*. neutralised lethality, neurotoxicity, cardiotoxicity and respiratory changes in experimental animals induced with *Naja kaouthia* venom (Chatterjee *et al*., 2006).

Herbal compounds are easily available, cheap, and stable at room temperature and can neutralize a range of venom antigens. However, in many cases, the whole plant extracts are more effective than the individual herbal compounds (Gomes *et al*., 2010). Traditionally, plant extracts are administered to victims of snake envenomation by: drinking plants extracts, topical application of plant sap or crushed/ground plant parts onto the bitten area, chewing of leaves or barks or a combination of two or more procedures. According to Laing *et al*., (1992) plant extracts or compounds with snake venom neutralisation properties are administered to experimental animal by any of the following three approaches:

i) Venom and herbal compounds mixed together then administered

ii) Herbal compounds administered then followed by venom

iii) Venom first administered then followed by herbal compounds

Of the three protocols, the third is similar to clinical conditions; however, the first approach has been found to be 2.5 times more effective in neutralising snake venom lethality (Laing *et al*., 1992).

Many hypotheses have been proposed to explain the mechanism of how plant compounds neutralize toxic venom components within the body. The mechanisms include: protein precipitation hypothesis (Vale *et al*., 2008), enzyme inactivation hypothesis (Hung *et al*., 2004), chelation hypothesis (Castro *et al*., 1999), adjuvant action hypothesis and anti-oxidant hypothesis (Gomes *et al*., 2007), combination hypothesis (Alam and Gomes, 2003) and many more. Among these hypotheses, the protein precipitation and enzyme inactivation hypotheses are more acceptable (Gomes *et al*., 2010). Plant secondary metabolites like flavonoides, xanthenes, quinonoid, polyphenols and terpenoids have been found to possess enzyme inhibiting and protein

binding properties that inhibit snake venom phospholipase A_2 (PLA_2) activities of both cobra and viper venom (Perumal *et al*., 2012).

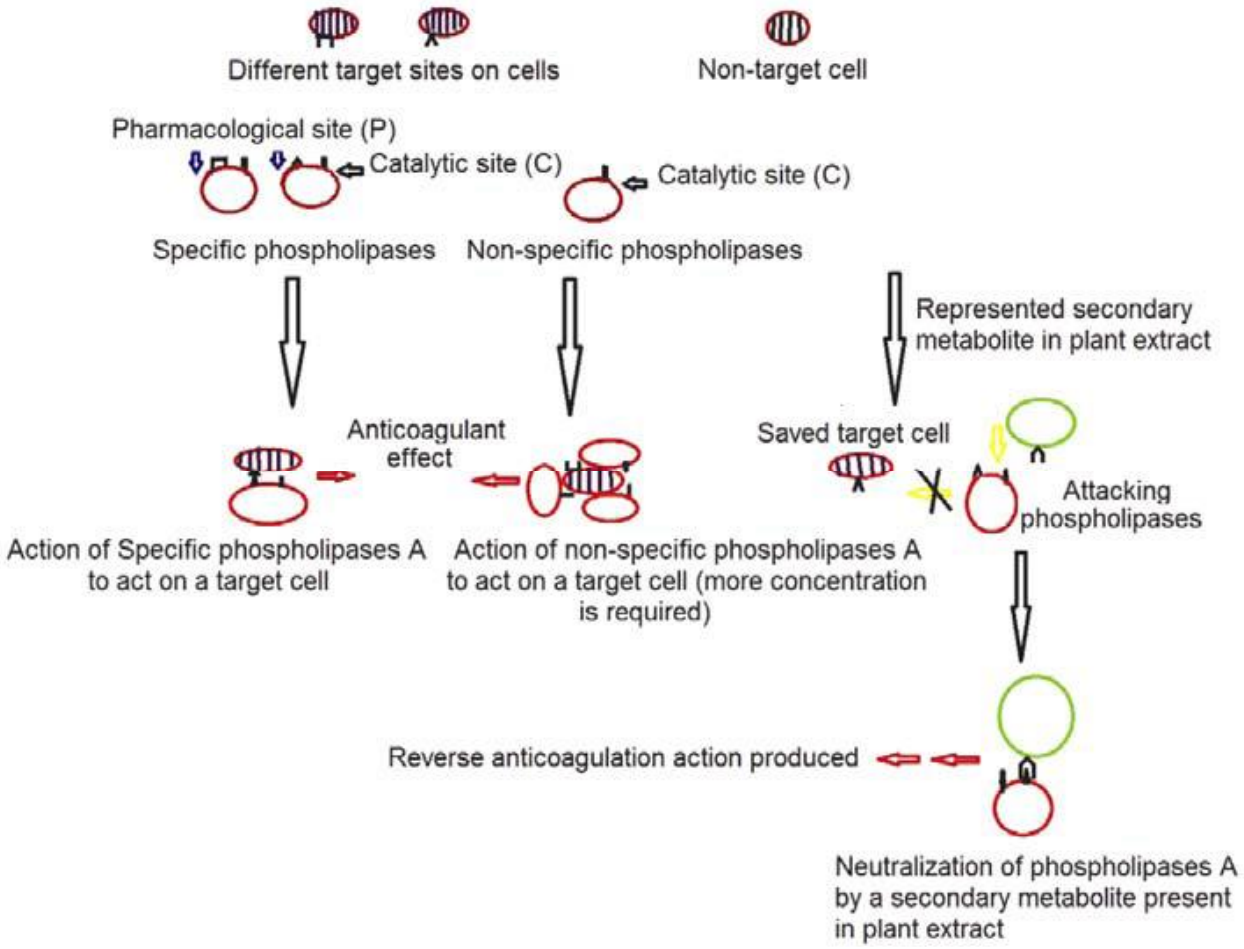

Figure 2.1: A proposed mechanism of action by phospholipase A_2 and its neutralization by secondary metabolites in medicinal plants (Kini, 2003)

2.7 Ethnobotanical description of the plants selected for the study

The following medicinal plants commonly used by traditional healers in Uasin-Gishu and Turkana counties were selected for the study and their ethnobotanical description are as summarized.

2.7.1 *Amaranthus spinosus* L.

The common name for *Amaranthus spinosus* L. is pigweed, spiny amaranth, prickly amaranth, thorny amaranth. Its local name in Nandi is Chepkerta. It is found in barns, waste places,

roadsides and pasture fields (Kawade *et al*., 2013). It is mainly found at an altitude of between 0 and 4653 meters above sea level (Chauhan and Johnson, 2009). It is used as diuretic, anti-diabetic, analgesic, anti-leprotic and in the treatment of bronchitis and piles (Husain *et al*., 2009). Its aqueous extract is also reported to show antimalarial activities *in vivo* and is able to stimulate the proliferation of β-lymphocytes *in vitro* (Azhar-ul-Haq *et al*., 2004). It is also reported to have anti-inflammatory and immunomodulatory properties (Ashok Kumar *et al*., 2011).

Plate 2.6: ***Amaranthus spinosus***

2.7.2 *Carissa spinarum Vahl*

The common name for *Carissa spinarum Vahl* is simple-spined num-num and it local name in Kalenjin is Tamuriekiat/Leketetuet. It grows in forests or forest edges and woodlands especially on rocky hillsides on a variety of soils and at altitude of 1500-2500 meters above sea level

(Kebenei *et al*., 2011). It is traditionally used to treat epilepsy, chest complains, headache, gonorrhea and syphilis as well as rheumatism, rabies and as a diuretic (Nedi *et al*., 2004). Its aqueous extracts show activity against herpes simplex virus (HSV) *in vitro* and *in vivo* (Tolo *et al*., 2010). Other folkloric uses of *C. edulis* include treatment for fever, hernia and sickle cell anaemia (Ya'u *et al*., 2008)

Plate 2.7: *Carissa spinarum* (photo courtesy of the researcher)

2.7.3 *Combretum molle*

Combretum molle local names include Keyo (Luo), Kiama/Muama (Kamba), Mwama/Mwawa (Taita), Mukhungula (Luhya), Murama (Meru) and Kemeliet (Nandi). It is found in woodlands and bushes, usually on rocky ridges and hillsides. A decoction obtained from boiling its roots is taken to kill hookworms (Ademola and Eloff, 2010), relieve stomach pains, fevers, backaches, general body swellings, and treat snake bite wounds, leprosy, dysentery and induces abortion

(Masoko *et al*., 2007). It is also mixed with porridge and taken for the treatment of sexually transmitted diseases such as syphilis and gonorrhea (Orwa *et al*., 2009). Its pounded leaves are soaked in water to make a decoction which is drunk to relief chest pains (Yeo *et al*., 2012). Both its leaves and roots show cytotoxicity effects against Cervical cancer (HeLa), Bladder cancer (T24), and Breast cancer (MCF7) (Fyhrquist *et al*., 2006). Some studies have also shown that *C. molle* has anti-asthmatic and antitussive activities (Grønhaug *et al*., 2008).

Plate 2.8: *Combretum molle*

2.7.4 *Cyperus articulatus*

Cyperus articulatus common names include adrue, guinea rush, jointed flat sedge and piripiri. Its local name in Turkana is Ekeriau. It is commonly found growing along river banks, in ponds, ditches, marshes and banks of lakes and canals. *Cyperus articulatus* produce compounds that are effective as anti-convulsants and is therefore used in calming epileptic seizures. In 19th and 20th

century, a drug called Adrue, made from roots of *C. articulatus* was sold in America as a digestive aid to help relieve nausea, gas, other digestive problems as well as morning sickness. At higher doses adrue was used to sedate anxious patients (Ngo Bum *et al*., 2003).

Plate 2.9: ***Cyperus articulatus***

2.7.5 *Rhynchosia usambarensis*

The local name for *Rhynchosia usambarensis* in Nandi is Cheperen. It is found on rocky hillsides among thickets of grass and bushes at an altitude of 1000-2400 meters above sea level.

Plate 2.10: ***Rhynchosia usambarensis***

2.8 Toxicity studies of plant extracts

Toxicology is an aspect of pharmacology which deals with the adverse effects of bioactive substances on living organisms whose aim is to establish scientifically the efficacy and safety of a drug in animal models (Spławiński *et al.*, 2006). Therefore, toxicological studies helps in making decisions as to whether a drug or natural remedies with potential health benefits should be adopted for clinical use or not (Pour *et al.*, 2011). Toxicological studies on animal models are categorized into three types depending on the duration of the drug's/compound's exposure to animals, that is : acute toxicity studies, sub-acute toxicity and chronic toxicity studies (Yeo *et al.*, 2012). Acute toxicity (lethal toxicity) is the ability of a chemical to cause ill effect within

minutes, hours or days up to two weeks after one administration or a 4h exposure of a chemical in air (Yeo *et al*., 2012). Usually, the LD_{50} (Lethal Dose 50%) also sometimes referred to as the Median Lethal Dose (MLD) is an index for acute toxicity and is essentially the amount of a substance that can cause death in half (50%) of a group of a particular animal species, usually mice or rats (Adumanya, 2014). The LD_{50} obtained at the end of a study is reported in relation to the route of administration of the test substance such as LD_{50} (oral), and LD_{50} (dermal). It is also expressed as the amount of a substance administered, such as milligrams per 1000 grams or per kilogram body weight. The most commonly used toxicity scales when comparing LD_{50} values are the Hodge and Sterner scale and the Gossein, Smith and Hodge scale (Ahmed, 2015).

Sub-acute toxicity studies involve administering repeated doses of a drug for a period of 14-30 days in sub-lethal quantity. It is used to determine the effect of a drug or a substance on serum biochemical parameters and hematological parameters as well as for histological changes. In chronic toxicity studies, a drug or substance is given in different dose levels for a period of 90 days to over a year to determine a drug's mutagenic and carcinogenic potential. Body weight change is one of the indices of toxicity assessment after an animal is exposed to toxic substances (Szczepańska *et al*., 2016; Pagano *et al*., 2007). However, Harizal *et al.* (2010) reported that a significant increase in the body weight of an animal is closely related to body fat accumulation rather than to the toxic effects of a drug and chemical. Everds *et al*. (2013) too suggested that reductions in an animal's body weight in toxicity studies may be due to normal physiological adaptation response by the animal model to plant extracts or chemical constituents, leading to low appetite and consequently lower caloric intakes. The relative organ weight studies are important in diagnosing whether the drug or substance administered inflicted injury to the organ

or not (Rajeh *et al*., 2012). The heart, kidney, liver, lungs and spleen are the primary organs affected by metabolic reactions caused by toxic substances (Dybing *et al*., 2008). The absolute organ weight is also a relatively sensitive indicator of nephrotoxicity. An increase in kidney weight, for instance either absolute or relative, is an indicator of nephrotoxicity (Swain *et al*., 2008). The hemopoietic system is a sensitive targets for toxic substances and equally an important index of pathological and physiological status in humans and animals (Abernethy *et al*., 2004). The most important haematological tests recommended in clinical pathology include: RBC count, total WBC count, platelet count, hemoglobin concentration and hematocrit concentration. Red blood cells count such as mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) are important in diagnosing anaemia.

Blood biochemical parameters such as blood plasma, creatinine, urea, uric acid, glucose, cholesterol, aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT) and electrolytes are also used to evaluate the toxic effects of chemical and non-chemical substances in animals (Ozer *et al*., 2010). Elevation of glucose, cholesterol, urea nitrogen, AST, ALT and albumin in serum blood of animals after administration of plant extracts shows that the administered herbal medicine has a toxic effect on the animal model. However, reduced levels of glucose, nitrogen, urea, and uric acid is an indication that the plant extracts have limited or no toxic effect on animals (Oyewole, 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Collection and identification of medicinal plants

Selected plants were collected from their natural habitats using their local names and their identity authenticated as per the International Code of Botanical Nomenclature by an acknowledged authority in taxonomy from the East African herbarium at the National Museums of Kenya. Plant parts collected from the selected plants are as follows: *Amarantus spinosus* (whole plant), *Carissa spinarum* (roots), *Combretum molle* (roots), *Cyperus articulatus* (rhizomes) and *Rhynchosia usambarensis* (whole plant). Coordinates for location of collection points for each plant was taken and recorded as follows: *Amarantus spinosus* E35.1381903N.5550996, *Carissa spinarum* E35.1471132N.6020413, *Combretum molle* E35.1393391N.553255, *Cyperus articulatus* E35.5872753N3.1115859 and *Rhynchosia usambarensis* E35.1386372N.5498066.

3.2 Preparation of plant extracts

One hundred grams (100 g) of shade dried powdered plant materials were mixed with 2000 ml double distilled water in 2500 ml screw caped conical flasks and kept on a water bath at 60ºC for 2h. The extracts were then filtered by using a muslin cloth and then re-filtered using Whatman filter paper No. 1. The filtrate was then stored in a refrigerator at 4^0C until they were freeze dried with a lyophilizer. Freeze dried materials were weighed and kept in a freezer at -20^0C until use.

3.3 Venom and experimental animals

Lyophilized snake venom powder of *Naja subfulva* were obtained from Bio-Ken snake farm, Watamu, Malindi and stored at 4ºC. Swiss albino mice of either sex (18-20 g) were used for efficacy and toxicity studies. The mice were bred under standard laboratory conditions of 12 hours exposure to light, 25 ± 2ºC temperature and 35-60 % humidity in the animal houses of the Departments of Biochemistry, Microbiology and Biotechnology of Kenyatta University and Medical Laboratory Sciences (MLS) of Jomo Kenyatta University of Agriculture and Technology. They were fed with standard mice pellets from Unga Feeds Limited and provided with water *ad libitum*.

3.4 *In vivo* venom-neutralization potency tests of the aqueous plant extracts

The only currently and globally validated means of assessing venom toxicity and antivenom neutralizing efficacy by manufacturers and regulatory authorities is by determining venom lethality (LD_{50}) and antivenom neutralizing capacity (ED_{50}) (Calvete *et al*., 2014).

3.4.1 Venom median lethal dose (LD_{50}) assay

The median lethal dose (LD_{50}) of *Naja subfulva* venom was assayed as per the method developed by Theakston and Reid (1983). Various doses of venom dissolved in 0.2 ml of physiological saline were injected into the tail vein of mice, using groups of 5 mice for each venom dose. The LD_{50} was estimated by probit analysis at 50% probability of deaths occurring within 24h of venom injection.

3.4.2 Aqueous extracts median effective dose (ED_{50}) assay

The venom neutralizing potential of aqueous extracts of the five selected plants was determined against $2LD_{50}$ ("challenge dose") of *Naja subfulva* venom. Various doses of plant extracts (100, 200, 300, 400 and 500 mg/kg body weight) were mixed with $2LD_{50}$ of *Naja subfulva* venom sample in a total of 0.2 ml and incubated at 37ºC for 30 minutes and injected intravenously into mice. Control mice received a mixture of venom "challenge dose" with physiological saline solution alone to confirm that the venom "challenge dose" induces 100 % lethality. Five mice were used for each antivenom dose. The median effective dose (ED_{50}) was calculated from the number of deaths occurring within 24h of injection of venom/antivenom mixture by probit analysis. The lower the ED_{50} value, the higher the neutralizing ability of the antivenom (Laing *et al*., 1992).

3.5 *In vitro* venom-neutralization potency tests of the aqueous plant extracts

3.5.1 Phospholipase A_2 activity

Phospholipase A_2 activity was determined using an indirect hemolytic assay on agarose-erythrocyte-egg yolk gel plate as per the methods described by Gutiérrez *et al*. (1988). Increasing doses of *N. subfulva* venom were added into 3mm wells in agarose gels (0.8 % in PBS, pH 8.1) containing 1.2 % sheep erythrocytes, 1.2 % egg yolk as a source of lecithin and 10 mM $CaCl_2$. Slides were then incubated at 37ºC overnight and the diameters of the hemolytic halos were measured. Control wells contained 15 µl of physiological saline. The minimum indirect hemolytic dose (MIHD) corresponds to a dosage of venom, which produces a hemolytic halo of 11 mm diameter. The efficacy of plant extracts in neutralizing the Phospholipase A_2 activity was determined by mixing a constant dose of venom (MIHD) with various doses of plant extracts and

incubating for 30 minutes at 37ºC. Aliquots of 10 µl of the mixtures were added to wells in agarose-egg yolk-sheep erythrocyte gels. Control samples contained venom without plant extracts. Plates were then incubated at 37ºC for 20 h. Neutralization was expressed as the plant extracts/venom's MIHD mixture that reduced the diameter of the hemolytic halo by 50 % when compared to the effect induced by venom's MIHD alone.

3.5.2 Procoagulant activity

Procoagulant activity was assayed according to the method described by Theakston and Reid (1983) and as modified by Laing *et al.* (1992). Various amounts of venom dissolved in 100 µL PBS (pH 7.2) was added to human citrated plasma at 37ºC. Coagulation time was recorded and minimum coagulation dose (MCD) determined as the venom dose which induced clotting of plasma within 60 seconds. Plasma incubated with PBS alone served as control. In neutralization assays, a constant dose of venom was mixed with various dilutions of plant extracts. The mixtures were then incubated for 30 minutes at 37ºC. 0.1 mL of the mixture was then added to 0.3 mL of citrated plasma and the clotting times recorded. In control tubes, plasma was incubated with either venom alone or plant extracts alone. Neutralization was expressed as effective dose (ED), defined as the plant extracts/venom dose (MCD) at which the clotting time increased three times when compared with clotting time of plasma incubated with two times MCD of venom alone.

3.6 Toxicity studies of plant extracts

Female albino mice (18-20 g) were individually identified and acclimatized to the laboratory conditions for seven days before the start of the study. The toxicity studies were conducted in

accordance with the methods described by Lorke (1983). It was conducted in two phases, sub-acute and acute phases. In the first phase (sub-acute), fifteen mice were randomly divided into three groups of five mice per group and each was given oral doses of 10, 300 and 1000 mg/kg body weight of the extract once daily for fourteen days. In addition, a fourth group of five mice was given physiological saline alone to serve as the control. In the second phase (acute), higher specific doses (1600, 2900 and 5000 mg/kg body weight) of the extract was administered once to nine mice (three mice per dose). The extracts were dissolved in physiological saline1` solution and given via intraperitoneal route. All mice were kept under the same conditions and observed for the first 4 critical hours, 72 hours and thereafter for 14 days. The number of deaths in each group within 24h was recorded and the final median lethal dose (LD_{50}) calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred). All surviving mice were sacrificed at the end of the 14 days and internal organs weighed. The organ: body weight ratios were calculated and compared with those of the control group. Hematological and biochemical parameters were also determined and organs prepared and fixed for histopathological studies.

3.6.1 Determination of body and organ weight

The body weight of each mouse under study was taken from day zero and thereafter every seven days during the dosing period up to and including the 14^{th} day, that is, day 0, 7 and 14.

3.6.2 Blood collection for hematological and biochemical analyses

At the end of the 14 days of the experimental period, all mice under study were weighed on a digital balance, anesthetized using diethyl ether and blood samples withdrawn by cardiac

puncture. Some of the blood samples obtained from each mouse was collected in separate test tubes with EDTA (Ethylene Diamine Tetra-acetic Acid) anticoagulant and the other placed in plain test tubes without EDTA.

Blood samples from test tubes containing EDTA were immediately processed for hematological parameters using Automated Hematological Analyzer (SYSMEX RX21, Japan). Red blood cell count (RBC), hemoglobin concentration (HB), hematocrit (PCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), platelet count (PLT), white blood cell count (WBC), neutrophils count (NEU), lymphocytes count (LYM), monocytes (MON), eosinophils count (EOS) and basophils count (BAS) were determined. Blood samples for biochemical analysis in plain test tubes were allowed to stand for 3 hours for complete clotting and then centrifuged at 5000 rpm for 15 minutes using a bench top centrifuge (HUMAX-K, HUMAN-GmbH, Germany) to obtain plasma. The plasma was then withdrawn and transferred into other clean vials and then kept at -20ºC until analysis for clinical biochemistry parameters was undertaken. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (GGT), total bilirubin (T-BIL), direct bilirubin (D-BIL), blood urea nitrogen (BUN), creatinine (CREAT), total bilirubin, and glucose (GLU) concentrations in the plasma sample were automatically analyzed by a Clinical Chemistry Autoanalyzer (AUTO LAB18 clinical chemistry analyzer, Italy).

3.6.3 Mice dissection and tissue collection

Following collection of blood samples, each mouse was euthanized using ether and sacrificed. Abdominal cavity was opened and the liver, kidney, heart, lungs, spleen and brain were carefully removed and cleared from any surrounding tissues using physiological saline. The organs were then put on a clean paper and weighed quickly on a digital balance. The percent organ to body weight was calculated using the formula described by Adewale *et al*. (2016).

$$\text{Relative organ weight} = \frac{\text{Absolute organ weight (g)}}{\text{Body weight of mouse on sacrifice day (g)}} \text{ X } 100$$

Randomly, the coronal section of some sample of the right and left kidney and some sample of transverse section of the lobe of liver were fixed in 10 % neutral buffered formalin solution for future histological processing.

3.7 Qualitative phytochemical screening of the aqueous plants extracts

The presence or absence of alkaloids, phenols, flavonoids, tannins, saponins, glycosides, phytosterols, resins, and bound anthraquinones in the aqueous plants extracts were carried out using standard methods as follows:

3.7.1 Alkaloids

The presence of alkaloids in the aqueous extracts was determined by first dissolving 20 mg of extract in 1 ml of methanol, filtering the mixture, transferring and boiling the extracts in 2 ml of 1% hydrochloric acid for 5 minutes and then finally adding 4-6 drops of Dragendorff's reagent into the extract. Formation of an orange precipitate indicated the presence of alkaloids (Surmaghi *et al*., 1992).

3.7.2 Tannins

Half a gram (0.5 g) of aqueous extract was dissolved in 2 ml of distilled water and filtered; two drops of ferric chloride was then added to the filtrate. A blue black precipitate was an indication of the presence of tannins (Jones and Kinghorn, 2005).

3.7.3 Cardiac glycosides

The presence of cardiac glycosides was determined by Keller-Kiliani test. A hundred milligrams (100 mg) of the aqueous plants extracts were treated with 1 ml of glacial acetic acid containing one drop of 5 % ferric chloride ($FeCl_3$) solution. To this solution, 1 ml of concentrated sulphuric acid was under-layered. The appearance of a brown ring at the interface of the two layers with the lower acidic layer turning blue green after standing for a few minutes was an indication of the presence of cardiac glycosides (Harborne, 1998).

3.7.4 Steroids

The presence of steroids was determined by Liebermann-Burchard reaction test. A chloroform solution of 0.5g of the aqueous plants extracts was treated with 0.5 ml of acetic anhydride and 2 drops of concentrated sulphuric acid added down the sides of the test-tube. A blue green ring result indicated the presence of sterols, while change of colour from pink to purple was an indication of presence of triterpenes (Hungund, 2014).

3.7.5 Saponins

The frothing test was used to determine the presence of saponins in the aqueous plants extracts. Five grams (0.5 g) of the aqueous plants extracts were shaken in 5 ml of distilled water and

allowed to stand for 10 minutes. A stable froth of more than 1.5 cm that persists for at least 30 minutes was an indication of the presence of saponins in the plant extracts under test (Kapoor *et al*., 1969).

3.7.6 Flavonoids and flavones

One gram (1 g) of each aqueous plant extract was dissolved in 10 ml distilled water and then filtered using Whatman filter No.1. 10 mg of magnesium turnings were added into 1 ml of the filtrate, followed by an addition of 0.05 ml concentrated sulphuric acid. The presence of magenta red within three minutes of testing was a confirmation of presence of flavonoids in the extracts, while orange colour was an indication of presence of flavones.

3.7.7 Terpenoids

Two millilitres (2 ml) of chloroform was mixed with 5 ml of each plant extract followed by an addition of 3 ml of concentrated sulphuric acid to form a layer. Formation of a reddish brown precipitate at the interface was an indication of the presence of terpenoids.

3.7.8 Phenols

The test for the presence of phenols in each of the aqueous plant extract was carried out by the Ferric chloride test. 5 % ferric chloride was added to a fraction of each extract and observed. Formation of a deep blue or black colour was an indication of presence of phenols in the extracts (Hungund, 2014).

3.8 Mineral element composition of the aqueous plants extracts

The extracts were analyzed for trace elements using Total Reflection X-ray Fluorescence system (TRXF). The system was used to determine content of sodium (Na), potassium (K), magnesium (Mg), calcium (Ca), manganese (Mn), chlorine (Cl), zinc (Zn), titanium (Ti), vanadium (V), iron (Fe), copper (Cu), gallium (Ga), bromine (Br), arsenic (As), selenium (Se), lead (Pb), rubidium (Rb), nickel (Ni), strontium (Sr), and uranium (U), in the lyophilized plant samples as described by Hagen (2007). The principle of TXRF is that atoms emit secondary X-rays called fluorescence radiation when irradiated. These fluorescence radiations are characteristic for a particular atom (element) and of specific energy which makes it possible for quantitative analyses (Desideri *et al*., 2011).

3.9 Data Management and Statistical analysis

Data was recorded in the Laboratory notebook and then entered into Excel Spreadsheet for cleaning after which it was exported to MINITAB 18 for statistical analysis. Results were expressed as the Mean±SD. One way analysis of variance (ANOVA) was used to assess any statistically significant differences ($p \leq 0.05$) among groups while Tukey HSD post hoc method was used to determine significant differences between groups. LD_{50} and ED_{50} were determined by probit analysis.

CHAPTER FOUR

RESULTS

4.1 Lethality of *Naja subfulva* venom and *in vivo* neutralization potency of the five aqueous plants extracts in mice

Lethality of *Naja subfulva* venom after its intravenous injection in mice through the tail vein is reported in Table 4.1. Results indicate that after intravenous injection of various dose of Naja subfulva venom in mice via the tail, the LD_{50} of the venom was found to be 0.99 mg/kg body weight (Appendix 1). The neutralization potency of aqueous extracts of *A. spinosus* (AS), *C. spinarum* (CS), *C. articulatus* (CA), *C. molle* (CM) and *R. usambarensis* (RU) were determined by mixing various doses of the plant extracts with $2LD_{50}$ (2 mg/kg body weight) of venom sample and incubating the mixture at 37ºC for 30 minutes prior intravenous injection into mice. Results indicate that the ED_{50} obtained for the aqueous extracts were 87.53, 72.11, 341.28, 119.24, and 113.38 mg/kg body weight for *A. spinosus* (AS), *C. spinarum* (CS), *C. articulatus* (CA), *C. molle* (CM) and *R. usambarensis* (RU), respectively (Table 4.2; Appendix 2-6).

Table 4.1: Median lethal dose (LD_{50}) of *Naja subfulva* venom in mice

Group	Dose (mg/kg body weight)	Mortality (24hr)	% Death	Log dose
1(Control)	0.2 ml saline	0/5	0	-
2	2.5	5/5	100	0.40
3	2.0	5/5	100	0.30
4	1.5	3/5	60	0.18
5	1.0	2/5	40	0.00
6	0.5	1/5	20	-0.30

Table 4.2: Effect of intravenous administration of preincubated mixture of $2LD_{50}$ (2 mg/kg body weight) *Naja subfulva* venom and the five aqueous plants extract to mice

Group	Dose (mg/kg body weight) extract	Mortality (24 hr)	% Survival	Log dose
	Amaranthus spinosus			
1 Control	0 (venom only)	5/5	0	-
2	100	2/5	60	2.00
3	200	1/5	80	2.30
4	300	1/5	80	2.48
5	400	0/5	100	2.60
6	500	0/5	100	2.70
	Carissa spinarum			
1 Control	0 (venom only)	5/5	0	-
2	100	2/5	60	2.00
3	200	1/5	80	2.30
4	300	1/5	80	2.48
5	400	1/5	80	2.60
6	500	0/5	100	2.70
	Combretum molle			
1 Control	0 (venom only)	5/5	0	-
2	100	3/5	40	2.00
3	200	1/5	80	2.30
4	300	1/5	80	2.48
5	400	1/5	80	2.60
6	500	0/5	100	2.70
	Cyperus articulatus			
1 Control	0 (venom only)	5/5	0	-
2	100	5/5	0	2.00
3	200	5/5	0	2.30
4	300	3/5	40	2.48
5	400	2/5	60	2.60
6	500	0/5	100	2.70
	Rhynchosia usambarensis			
1 Control	0 (venom only)	5/5	0	-
2	100	3/5	40	2.00
3	200	1/5	80	2.30
4	300	2/5	60	2.48
5	400	2/5	60	2.60
6	500	1/5	80	2.70

4.2 *In vitro* venom neutralization potency of the five aqueous plants extracts

4.2.1 Phospholipase A_2 activity

Results of the phospholipase A2 assay demonstrated that 1.5 mg/kg body weight dose of *N. subfulva* venom produced 11 mm diameter hemolytic halos; this was considered as 1 IU/10 µg and as the minimum hemolytic dose (MHD). This demonstrates that *N. subfulva* venoms have the enzyme (phospholipase A_2) that has the ability to lyse sheep RBC upon incubation with different concentrations of aqueous extracts of *A. spinosus* (AS), *C. articulatus* (CA), *C. spinarum* (CS), *C. molle* (CM), and *R. usambarensis* (RU) from 100-500 mg/kg body weight (Tables 4.3). Results indicate that *A. spinosus* (AS) prevented hemolysis by 57.3, 58.2, 60.0, 64.5 and 66.4 % at 100, 200, 300, 400 and 500 mg/kg body weight, respectively; *C. spinarum* (CS) prevented hemolysis by 42.7, 44.5, 42.7, 48.2, and 50 % at 100, 200, 300, 400 and 500 mg/kg body weight, respectively; *C. molle* (CM) prevented hemolysis by 41.8, 42.9, 43.6, 46.4 and 48.2 % at 100, 200, 300, 400 and 500 mg/kg body weight, respectively; *C. articulatus* (CA) prevented hemolysis by 30.9, 32.7, 32.7, 36.4, 40.0 % at 100, 200, 300, 400 and 500 mg/kg body weight, respectively; and *R. usambarensis* (RU) prevented hemolysis by 1.0, 21.8, and 25.5 % at 300, 400, and 500 mg/kg body weight, respectively (Table 4.4). The order of phospholipase A2 activity of the aqueous extract was *A. spinosus* (AS) > *C. spinarum* (CS) > *C. molle* (CM) > *C. articulatus* (CA) > *R. usambarensis* (RU).

Table 4.3: Effect of *N. subfulva* venom induced hemolysis on sheep RBCs plate by PLA_2 activity

Venom(mg)	Haloes (mm)
0 (control)	3.00±0.00
2.5	13.00±0.20
2.0	12.10±0.20
1.5	11.03±0.25
1.0	7.03±0.12
0.5	6.03±0.06

Table 4.4: Effect of aqueous extract of *A. spinosus, C. articulatus, C. spinarum, C. molle* and *R. usambarensis* on *N. subfulva* venom induced hemolysis on sheep RBCs plate by PLA2 activity

Treatment	AS		CA		CS		CM		RU	
Venom 1.5 mg plus extract	Hallos in mm	% inhibition	Hallos in mm	% inhibition	Hallos in mm	% inhibition	Hallos in mm	% inhibition	Hallos in mm	% inhibition
100	4.7±0.08	57.30	7.6±0.13	30.90	6.3±0.21	42.70	6.4±0.08	41.80	12.17±0.24	-10.60
200	4.6±0.06	58.20	7.4±0.08	32.70	6.1±0.14	44.50	6.3±0.13	42.90	11.33±0.24	-3.00
300	4.4±0.10	60.00	7.4±0.06	32.70	6.3±0.31	42.70	6.2±0.10	43.60	10.9±0.08	1.00
400	3.9±0.13	64.50	7.0±0.08	36.40	5.7±0.17	48.20	5.9±0.08	46.40	8.6±0.14	21.80
500	3.7±0.10	66.40	6.6±0.13	40.00	5.5±0.08	50.00	5.7±0.13	48.20	8.2±0.29	25.50

Results are expressed as Mean ± SD of 5 mice per dose group.

4.2.2 Procoagulant activity

Results indicate that *Naja subfulva* venoms at doses of 500 mg/kg body weight had no significant procoagulant activity using the method described by Theakston and Reid (1983).

4.3 *In vivo* single dose (acute) and repeated dosing (subacute) toxicity studies of the five aqueous plants extracts in mice

Table 4.5 depicts the effects of oral administration of the five aqueous plants extracts to mice as single high doses (acute toxicity) and repeated therapeutic doses (subacute toxicity) for 14 days. Results indicate that oral administration of aqueous extracts to mice as single high doses of 1600, 2900 and 5000 mg/kg body weight caused no death within the 14 days observation period of the study for all the three tested doses. The LD_{50} of these five aqueous plants extracts in mice model was therefore greater than 5000 mg/kg body weight. For the repeated dosing, oral administration of the five aqueous plants extracts to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight caused no death within the study period for all the tree tested doses.

Table 4.5: Acute and subacute toxicity of oral administration of the five aqueous plants extracts to mice at therapeutic and high doses observed for 14 days

Toxicity study type	Dose (mg/kg body weight)	Deaths within 24 hr
Amaranthus spinosus		
Acute (single dose)	Control	0/3
	1600	0/3
	2900	0/3
	5000	0/3
Subacute (repeated dosing)	Control	0/5
	10	0/5
	300	0/5
	1000	0/5
Cyperus articulatus		
Acute (single dose)	Control	0/3
	1600	0/3
	2900	0/3
	5000	0/3
Subacute (repeated dosing)	Control	0/5
	10	0/5
	300	0/5
	1000	0/5
Carissa spinarum		
Acute (single dose)	Control	0/3
	1600	0/3
	2900	0/3
	5000	0/3
Subacute (repeated dosing)	Control	0/5
	10	0/5
	300	0/5
	1000	0/5
Combretum molle		
Acute (single dose)	Control	0/3
	1600	0/3
	2900	0/3
	5000	0/3
Subacute (repeated dosing)	Control	0/5
	10	0/5
	300	0/5
	1000	0/5
Rhynchosia usambarensis		
Acute (single dose)	Control	0/3
	1600	0/3
	2900	0/3
	5000	0/3
Subacute (repeated dosing)	Control	0/5
	10	0/5

300	0/5
1000	0/5

4.3.1 Effects of oral administration of the five aqueous plant extracts to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight on body weight, average body weight change and average weekly body weight change, and mortality

The effects of oral administration of aqueous extracts of *A. spinosus* (AS), *C. articulatus* (CA), *C. spinarum* (CS), *C. molle* (CM) and *R. usambarensis* (RU) to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight on body weight and average weekly body weight change are reported in Table 4.6. Results indicate that there were no deaths observed in any of the extract treated mice at 10, 300 and 1000 mg/kg body weight for the 14 days of subacute toxicity study. However, the extract-treated mice gained less body weight at the end of experimentation compared to that of the normal control mice in a dose dependent manner. For example, the average body weight of mice treated with extracts of *A. spinosus* (AS) was increased to 2.68 g (77.23%), 1.30 g (37.40%), and 0.30 g (8.65%); *C. articulatus* (CA) was increased to 3.06 g (76.50%), 1.53 g (38.25%), and 0.67 g (16.75%); *C. spinarum* (CS) was increased to 3.03 g (68.09%), 1.44 g (32.36%), and -0.48 g (-10.79%); *C. molle* (CM) was increased to 2.33 g (75.90%), 1.83 g (59.61%), and 0.50 g (16.29%); and *R. usambarensis* (RU) was increased to 2.83 g (65.97%), 2.41 g (56.18%), and 0.14 g (3.26%) at 10, 300 and 1000 mg/kg body weight, respectively, which were significantly lower compared to that of the normal control mice at the 14^{th} day of the experimental period. In addition, the average weekly body weight change of the experimental mice significantly decreased with the increase of the treatment dose from 10 to 1000 mg/kg body weight for all the five aqueous plants extracts. The percent decrease in average weekly body weight of experimental mice caused by the aqueous extracts were 23.79, 62.11 and 92.51% for *A. spinosus*; 23.60, 61.42 and 83.15 % for *C. articulatus*; 27.96, 63.82 and 101.32 %

for *C. spinarum*; 44.44, 55.91 and 88.17 % for *C. molle* and 22.22, 54.51 and 96.18 % for *R. usambarensis* at 10, 300 and 1000 mg/kg body weight doses, respectively.

Table 4.6: Effects of oral administration of the five aqueous plants extracts to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight on body weight, average body weight change and average weekly body weight change

Plant extract	Dose (mg/kg body weight)	Time (Days)			Average body weight change (%)	Average weekly body weight change
		0	7	14		
Amaranthus spinosus	**Control**	**20.36±1.65**	**22.76±1.49**	**24.90±1.49**	**3.47±0.37 (100)^{a}**	**2.27±0.20^{a}**
	10	20.26±1.07	22.16±1.13	23.72±1.08	**2.68±0.19** (**77.23**)^{b}	**1.73±0.12^{b}**
	300	19.90±1.24	20.78±1.27	21.60±1.08	**1.30±0.08 (37.47)^{c}**	**0.86±0.09^{c}**
	1000	**20.00±1.16**	**20.26±1.59**	**20.34±1.48**	**0.30±0.51 (8.65)^{d}**	**0.17±0.24^{d}**
Cyperus articulatus	**Control**	**24.20±1.02**	**26.86±1.22**	**29.54±1.29**	**4.00±0.20** (**100**)^{a}	**2.67±0.14^{a}**
	10	23.80±1.19	25.84±1.24	27.88±1.25	**3.06±0.16** (**76.50**)^{b}	**2.04±0.07^{b}**
	300	23.98±1.09	24.98±1.05	26.04±1.05	**1.53±0.23** (**38.25**)^{c}	**1.03±0.15^{c}**
	1000	**23.32±1.45**	**23.76±1.44**	**24.22±1.51**	**0.67±0.13 (16.75)^{d}**	**0.45±0.08^{d}**
Carissa spinarum	**Control**	**19.33±1.45**	**22.46±1.84**	**25.20±1.35**	**4.45±0.55** (**100**)^{a}	**3.04±0.37^{a}**
	10	17.14±1.99	18.82±2.12	21.52±2.64	**3.03±1.05** (68.09)^{b}	**2.19±0.67^{b}**
	300	19.08±1.27	19.94±1.20	21.02±1.23	**1.44±0.63** (**32.36**)^{c}	**1.10±0.22^{c}**
	1000	**18.50±1.90**	**17.64±2.04**	**18.40±1.97**	**-0.48±0.59 (-10.79)^{d}**	**-0.04±0.26^{d}**
Combretum molle	**Control**	**23.20±1.30**	**25.70±1.29**	**28.18±1.36**	**3.07±1.41** (**100**)^{a}	**2.79±0.36^{a}**
	10	24.00±1.00	25.56±1.07	27.1±1.12	**2.33±0.21** (**75.90**)^{a}	**1.55±0.10^{b}**
	300	24.00±0.70	25.20±0.71	26.46±0.71	**1.83±0.08** (**59.61**)^{a}	**1.23±0.03^{b}**
	1000	**23.60±1.14**	**23.04±1.03**	**24.30±1.01**	**0.50±0.09 (16.29)^{b}**	**0.33±0.05^{c}**
Rhynchosia usambarensis	**Control**	**20.38±1.59**	**23.20±1.67**	**26.14±1.64**	**4.29±0.25** (**100**)^{a}	**2.88±0.14^{a}**
	10	21.44±1.50	23.20±1.55	25.34±1.37	**2.83±0.28 (65.97)^{b}**	**2.24±0.64^{a}**
	300	21.94±1.66	23.54±1.77	25.16±1.89	**2.41±0.33** (**56.18**)^{b}	**1.31±0.67^{b}**
	1000	**22.28±1.39**	**22.34±1.44**	**22.52±1.45**	**0.14±0.10 (3.26)^{c}**	**0.11±0.04^{c}**

Results are expressed as mean ± SD of five mice for each dose for each plant extract. Values with the same superscript down the column for each extract dose treatments are not significantly different from each other at $p < 0.05$ by ANOVA followed by Tukey's post hoc test.

4.3.2 Effects of oral administration of the five aqueous plants extracts to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight on percent organ to body weight and organ weight

The effects of oral administration of the five aqueous plants extracts to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight on percent organ to body weight and organ weight are reported in Table 4.7. [Treatment of mice with *A. spinosus* extract at 1000 mg/kg body weight caused a significant increase in the percent organ to body weight of the brain and lungs by 15.11 and 8.33%, respectively, compared to that of the normal control mice which was similar to that caused by the extract-treated mice at 10 and 300 mg/kg body weight doses. *A. spinosus* extract-treated mice at 300 and 1000 mg/kg body caused a significant and similar increase in the percent organ to body weight of the brain and lungs. Treatment of mice with *A. spinosus* extract at 1000 mg/kg body weight caused a significant increase in the percent organ to body weight of the liver compared to that of the normal control mice which was similar to that of the extract-treated mice at 10 mg/kg body weight dose. Further, treatment of mice with *A. spinosus* extract at 10 and 300 mg/kg body weight caused a significant and similar increase in the percent organ to body weight of the liver. *A. spinosus* extract-treated mice at 300 and 1000 mg/kg body weight caused a significant and similar increase in the percent organ to body weight of the liver. Treatment of mice with *A. spinosus* extract at 10 mg/kg body weight caused a significant increase in the percent organ to body weight of the kidney compared to that of the normal control mice which was similar to that of the extract-treated mice at 300 and 1000 mg/kg body weight doses. Treatment of mice with *A. spinosus* extract at 300 mg/kg body weight caused a significant increase in the percent organ to body weight of the spleen compared to that of the normal control mice which was similar to that of the extract-treated mice at 10 mg/kg body weight dose. *A. spinosus* extract-treated mice at 10 and 1000 mg/kg body weight doses caused a significant and

similar increase in the percent organ to body weight of the spleen. *A. spinosus* extract-treated mice at 300 and 1000 mg/kg body weight caused a significant and similar increase in the percent organ to body weight of the spleen. Treatment of mice with an extract of *A. spinosus* at 10, 300 and 1000mg/kg body weight caused a significant and similar increase in the percent organ to body weight of the heart compared to that of the normal control mice.

Treatment of mice with *C. articulatus* extract at 1000 mg/kg body weight dose significantly increased the percent organ to body weight of the brain and liver by 16.67 and 13.61 %, respectively, compared to that of the normal control mice which was similar to that of the extract-treated mice at 10 mg/kg body weight dose. *C. articulatus* extract-treated mice at 10 and 300 mg/kg body weight caused a significant and similar increase in the percent organ to body weight of the brain and liver. *C. articulatus* extract-treated mice at 300 and 1000 mg/kg body weight doses caused a significant and similar increase in the percent organ to body weight of the brain and liver.

Treatment of mice with *C. articulatus* extract at 300 mg/kg body weight dose caused a significant increase in the percent organ to body weight of the kidney by 7.14 % compared to that of the normal control mice which was similar to that of the extract-treated mice at 10 and 1000 mg/kg body weight doses. *C. articulatus* extract-treated mice at 10, 300 and 1000 mg/kg body weight doses caused a significant and similar increase in the percent organ to body weight of the kidney.

Treatment with *C. articulatus* extract at 1000 mg/kg body weight caused a significant decrease and increase in the percent organ to body weight of the lungs and spleen by 2.3 and 10.11 %, respectively, compared to that of the normal control mice which was similar to that of the extract-treated mice at 10 and 300 mg/kg body weight doses. *C. articulatus* extract-treated mice at 300 and 1000 mg/kg body weight doses caused a significant and similar decrease and increase in the percent organ to body weight of the lungs and spleen, respectively.

Treatment of mice with *C. articulatus* extract at 300 and 1000 mg/kg body weight doses caused a significant increase in the percent organ to body weight of the heart by 9.76 % compared to the normal control mice which was similar to the extract-treated mice at 10 mg/kg body weight dose. *C. articulatus* extract-treated mice at 10, 300 and 1000 mg/kg body weight doses caused a significant and similar increase in the percent organ to body weight of the heart.

Treatment of mice with *C. spinarum* extract at 1000 mg/kg body weight dose caused a significant increase in the percent organ to body weight of the brain, lungs and spleen by 27.87, 17.46 and 25.74 %, respectively, compared to that of the normal control mice which was similar to that of the extract-treated mice at 10 and 300 mg/kg body weight doses. *C. spinarum* extract-treated mice at 10, 300, and 1000 mg/kg body weight doses caused a significant and similar increase in the percent organ to body weight of the brain, lungs and spleen, respectively.

Treatment of mice with *C. spinarum* extract-treated mice at 1000 mg/kg body weight dose caused a significant increase in the percent organ to body weight of the liver by 34.47 % compared to that of the normal control mice which was similar to that of the extract-treated mice

at 10 and 300 mg/kg body weight doses. *C. spinarum* extract-treated mice at 300 and 1000 mg/kg body weight doses caused a significant and similar increase in the percent organ to body weight of the liver.

Treatment of mice with *C. spinarum* extract at 1000 mg/kg body weight dose caused a significant increase in the percent organ to body weight of the kidney by 3.41 % compared to that of the normal control mice which was similar to that of the extract-treated mice at 10 and 300 mg/kg body weight doses. *C. spinarum* extract-treated mice at 10 and 300 mg/kg body weight doses caused similar effects to the percent organ to body weight of the kidney which were significantly lower than that of the extract-treated mice at 1000 mg/kg body weight dose. Treatment of mice with *C. spinarum* extract at 10, 300 and 1000 mg/kg body weight doses caused similar effects to the percent organ to body weight of the heart which was significantly greater (31.11 %) than that of the normal control mice.

Treatment of mice with *C. molle* extract at 1000 mg/kg body weight dose caused a significant increase in the percent organ to body weight of the brain and liver by 10.65 and 13.63 %, respectively, compared to that of the normal control mice which was similar to that of the extract-treated mice at 10 and 300 mg/kg body weight doses. *C. molle* extract-treated mice at 10 and 300 mg/kg body weight caused similar effects to the percent organ to body weight of the brain and liver, respectively, which were significantly lower than that of the extract-treated mice at 1000 mg/kg body weight dose.

Treatment of mice with *C. molle* extract at 10, 300 and 1000 mg/kg body weight doses caused similar effects to the percent organ to body weight of the kidney which was comparable to that of the normal control mice. Treatment of mice with *C. molle* extract at 1000 mg/kg body weight dose caused a significant increase in the percent organ to body weight of the lungs by 5.88 % compared to that of the normal control mice which was similar to that of the extract-treated mice at 10 and 300 mg/kg body weight doses. *C. molle* extract-treated mice at 10 and 1000 mg/kg body weight doses caused similar effects to the percent organ to body weight of the lungs which were significantly higher than that of the extract-treated mice at 300 mg/kg body weight dose.

Treatment of mice with *C. molle* extract at 300 and 1000 mg/kg body weight doses caused similar effects to the percent organ to body weight of the spleen which were comparable to that of the normal control mice. *C. molle* extract-treated mice at 10 and 300 mg/kg body weight doses caused similar effects to the percent organ to body weight of the spleen which were comparable to that of the normal control mice. *C. molle* extract-treated mice at 10 and 300 mg/kg body weight doses caused similar effects to the percent organ to body weight of the spleen which was significantly higher than the extract-treated mice at 1000 mg/kg body weight dose.

Treatment of mice with *C. molle* extract at 1000 mg/kg body weight dose caused a significant increase in the percent organ to body weight of the heart by 6.98 % compared to that of the normal control mice which was similar to that of the extract-treated mice at 10 and 300 mg/kg body weight doses. *C. molle* extract-treated mice at 10 and 300 mg/kg body weight doses caused similar effects to the percent organ to body weight of the heart which was significantly lower than that of the extract-treated mice at 1000 mg/kg body weight dose.

Treatment of mice with *R. usambarensis* extract at 10, 300 and 1000 mg/kg body weight doses caused similar effects to the percent organ to body weight of the brain and kidney which were comparable to those of the normal control mice. Treatment of mice with *R. usambarensis* extract at 1000 mg/kg body weight dose caused a significant increase in the percent organ to body weight of the liver by 14.83 % compared to that of the normal control mice which was similar to that of the extract-treated mice at 10 and 300 mg/kg body weight.

Treatment of mice with *R. usambarensis* extract at 1000 mg/kg body weight caused a significant increase in the percent organ to body weight of the lungs compared to that of the extract-treated mice at 10 and 300 mg/kg body weight doses which were similar to those of the normal control mice. *R. usambarensis* extract-treated mice at 1000 mg/kg body weight dose caused similar effects to the percent organ to body weight of the lungs which were comparable to that of the normal control mice. *R. usambarensis* extract-treated mice at 10 and 300 mg/kg body weight caused similar effects to the percent organ to body weight of the lungs which were significantly lower than that of the extract-treated mice at 1000 mg/kg body weight dose.

Treatment of mice with *R. usambarensis* extract at 300 and 1000 mg/kg body weight doses caused similar effects to the percent organ to body weight of the spleen which are comparable to those of the normal control mice. *R. usambarensis* extract-treated mice at 10, and 300 mg/kg body weight doses caused similar effects to the percent organ to body weight of the spleen which was comparable to that of the normal control mice. *R. usambarensis* extract-treated mice at 1000 mg/kg body weight dose caused a significant increase in the percent organ to body weight of the spleen compared to that of extract-treated mice at 10 mg/kg body weight dose.

Table 4.7: Effects of oral administration of the five aqueous plants extracts to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight on percent organ to body weight and organ weight

Plant extract	Dose (mg/kg body weight)	Percent organ to body weight (body weight)					
		Brain	**Liver**	**Kidney**	**Lungs**	**Spleen**	**Heart**
Amaranthus spinosus	Control	**2.89±0.13^{B}** (0.72±0.01)^{a}	**6.58±0.35^{C}** (1.63±0.01)^{a}	**2.08±0.09^{A}** (0.52±0.01)^{a}	**1.32±0.05^{B}** (0.33±0.01)^{a}	**1.02±0.07^{C}** (0.25±0.02)^{ab}	**0.48±0.03^{B}** (0.12±0.01)^{a}
	10	**3.04±0.13^{B}** (0.72±0.01)^{a}	6.84±0.27^{BC} (1.62±0.01)^{ab}	**1.86±0.05^{B}** (0.44±0.01)^{b}	1.30±0.04^{B} (0.31±0.01)^{b}	1.06±0.08^{BC} (0.25±0.01)^{ab}	0.52±0.01^{A} (0.12±0.01)^{a}
	300	**3.15±0.13^{AB}** (0.68±0.01)^{b}	7.43±0.33^{AB} (1.60±0.02)^{bc}	**2.03±0.10^{A}** (0.40±0.04)^{b}	1.37±0.03^{AB} (0.30±0.02)^{b}	**1.22±0.05^{A}** (0.26±0.01)^{a}	0.52±0.02^{A} (0.11±0.01)^{a}
	1000	**3.33±0.20^{A}** (0.67±0.01)^{b}	**7.88±0.52^{A}** (1.60±0.01)^{c}	2.05±0.09^{A} (0.42±0.01)^{b}	1.43±0.07^{A} (0.29±0.01)^{b}	**1.16±0.08^{AB}** (0.24±0.01)^{b}	0.54±0.01^{A} (0.11±0.01)^{a}
Cyperus articulatus	Control	**2.52±0.08^{C}** (0.74±0.01)^{a}	**5.60±0.19^{C}** (1.65±0.02)^{a}	**1.82±0.06^{B}** (0.54±0.01)^{a}	**1.30±0.03^{B}** (0.33±0.01)^{a}	**0.89±0.02^{B}** (0.26±0.01)^{a}	**0.41±0.02^{B}** (0.12±0.00)^{a}
	10	**2.64±0.10^{BC}** (0.74±0.01)^{ab}	**5.88±0.24^{BC}** (1.64±0.02)^{ab}	**1.90±0.07^{AB}** (0.53±0.01)^{a}	**1.18±0.02^{B}** (0.33±0.01)^{a}	**0.91±0.01^{B}** (0.25±0.01)^{a}	0.42±0.01^{AB} (0.12±0.01)^{b}
	300	**2.78±0.09^{AB}** (0.72±0.01)^{bc}	**6.26±0.23^{AB}** (1.63±0.01)^{ab}	1.95±0.04^{A} (0.51±0.02)^{a}	**1.20±0.04^{AB}** (0.31±0.01)^{b}	**0.92±0.04^{AB}** (0.24±0.01)^{b}	0.45±0.01^{A} (0.12±0.01)^{b}
	1000	2.94±0.14^{A} (0.71±0.01)^{c}	**6.68±0.40^{A}** (1.61±0.01)^{b}	**1.89±0.08^{AB}** (0.46±0.04)^{a}	**1.27±0.08^{A}** (0.31±0.01)^{b}	0.98±0.07^{A} (0.24±0.01)^{b}	0.45±0.03^{A} (0.11±0.00)^{b}
Carissa spinarum	Control	**2.87±0.12^{B}** (0.72±0.01)^{a}	**6.47±0.32^{B}** (1.63±0.01)^{a}	**2.05±0.08^{AB}** (0.52±0.11)^{a}	**1.26±0.04^{B}** (0.32±0.01)^{a}	**1.01±0.05^{B}** (0.25±0.01)^{a}	**0.45±0.04^{B}** (0.12±0.01)^{a}
	10	**3.25±0.31^{AB}** (0.69±0.03)^{ab}	**7.46±0.86^{B}** (1.60±0.04)^{a}	1.97±0.17^{AB} (0.42±0.03)^{b}	1.36±0.10^{AB} (0.29±0.02)^{b}	**1.16±0.17^{AB}** (0.25±0.01)^{a}	0.53±0.04^{A} (0.11±0.01)^{a}
	300	**3.26±0.20^{AB}** (0.68±0.01)^{ab}	**7.60±0.42^{AB}** (1.60±0.01)^{a}	1.96±0.09^{B} (0.41±0.01)^{b}	**1.39±0.07^{AB}** (0.29±0.01)^{b}	**1.18±0.07^{AB}** (0.25±0.02)^{a}	0.53±0.03^{A} (0.11±0.01)^{a}
	1000	**3.67±0.31^{A}** **(0.67±0.02)^{b}**	**8.70±0.05^{A}** (1.60±0.01)^{a}	2.21±0.19^{A} (0.40±0.01)^{b}	**1.48±0.10^{A}** (0.27±0.01)^{b}	**1.27±0.11^{A}** (0.23±0.01)^{a}	**0.59±0.05^{A}** (0.11±0.01)^{a}
Combretum molle	Control	**2.63±0.10^{B}** (0.74±0.01)^{a}	**5.87±0.25^{B}** (1.65±0.01)^{a}	**1.90±0.07^{A}** (0.53±0.01)^{a}	**1.19±0.04^{B}** (0.33±0.01)^{a}	**0.94±0.04^{AB}** (0.26±0.05)^{a}	**0.43±0.02^{B}** (0.12±0.00)^{a}
	10	2.71±0.10^{B} (0.73±0.01)^{a}	**6.02±0.25^{B}** (1.63±0.01)^{b}	1.95±0.08^{A} (0.53±0.01)^{a}	1.20±0.03^{AB} (0.32±0.01)^{a}	0.90±0.02^{B} (0.24±0.01)^{b}	0.43±0.01^{B} (0.12±0.01)^{a}
	300	2.75±0.06^{B}	**6.16±0.14^{B}**	1.96±0.04^{A}	1.16±0.02^{B}	0.92±0.01^{AB}	0.44±0.01^{AB}

		(0.73±0.01)^{a}	(1.63±0.01)^{b}	(0.52±0.01)^{a}	(0.31±0.01)^{b}	(0.24±0.01)^{b}	(0.12±0.01)^{a}
	1000	**2.91±0.07^{A}**	**6.67±0.25^{A}**	1.95±0.07^{A}	**1.26±0.05^{A}**	0.96±0.03^{A}	0.46±0.01^{A}
		(0.71±0.01)^{b}	(1.62±0.01)^{b}	(0.47±0.03)^{b}	(0.31±0.01)^{b}	(0.23±0.01)^{b}	(0.11±0.01)^{a}
Rhynchosia usambarensis	Control	**2.80±0.12^{A}**	**6.27±0.03^{B}**	**2.00±0.07^{A}**	**1.24±0.05^{AB}**	**0.99±0.05^{AB}**	**0.45±0.02^{B}**
		(0.73±0.02)^{a}	(1.63±0.02)^{a}	(0.52±0.02)^{a}	(0.32±0.01)^{a}	(0.26±0.01)^{a}	(0.12±0.01)^{a}
	10	2.84±0.12^{A}	6.36±0.31^{B}	1.97±0.08^{A}	1.21±0.07^{B}	0.94±0.04^{B}	0.46±0.03^{B}
		(0.72±0.01)^{a}	(1.62±0.01)^{a}	(0.50±0.04)^{a}	(0.31±0.08)^{a}	(0.24±0.01)^{a}	(0.12±0.01)^{a}
	300	2.86±0.15^{A}	**6.49±0.41^{B}**	1.93±0.20^{A}	1.24±0.07^{B}	0.98±0.06^{AB}	0.46±0.02^{AB}
		(0.72±0.02)^{a}	(1.63±0.02)^{a}	(0.49±0.05)^{ab}	(0.31±0.01)^{a}	(0.25±0.02)^{a}	(0.12±0.01)^{a}
	1000	**3.06±0.26^{A}**	**7.20±0.44^{A}**	1.91±0.11^{A}	1.36±0.07^{A}	1.10±0.10^{A}	0.51±0.03^{A}
		(0.70±0.02)^{a}	(1.62±0.01)^{a}	(0.43±0.03)^{b}	(0.31±0.01)^{a}	(0.25±0.02)^{a}	(0.11±0.01)^{a}

Results are expressed as mean ± SD of five mice for each dose for each plant extract. Values with the same superscript down the column for each extract dose treatments are not significantly different from each other at $p < 0.05$ by ANOVA followed by Tukey's post hoc test.

4.3.3 Effects of oral administration of the five aqueous plants extracts to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight on hematological parameters

The effects of oral administration of the five aqueous plants extracts at 10, 300 and 1000 mg/kg body weight to normal mice daily for fourteen days on hematological parameters are reported in Table 4.8. Results indicate that oral administration of aqueous extracts of *A. spinosus, C.* articulatus and *R. usambarensis* to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight caused a non-significant effect to the levels of RBC ($x10^{12}$/L), HB (g/dL), PCV (%), MCH (pg), MCHC (g/dL), MCV (fL) and PLT ($x10^{9}$/L) compared to that of the normal control mice. Interestingly, oral administration of aqueous extracts of *C. spinarum* to mice daily for 14 days at 10 and 300 mg/kg body weight significantly decreased the levels of RBC ($x10^{12}$/L), HB (g/dL), and PCV (%) compared to that of the normal control mice which was similar to that of the extract-treated mice at 1000 mg/kg body weight dose. Treatment of mice with *C. spinarum* extract at 10, 300 and 1000 mg/kg body weight caused a non-significant effect on the levels of MCH (pg), MCHC (g/dL), MCV (fL) and PLT ($x10^{9}$/L) compared to that of the normal control mice. Oral administration of aqueous extracts of *C. molle* to mice daily at 10 and 1000 mg/kg body weight significantly increased the level of MCHC (g/dL) compared to that of the normal control mice which was similar to that of the extract-treated mice at 300 mg/kg body weight dose. Treatment of mice with *C. molle* extract at 10, 300 and 1000 mg/kg body weight caused a non-significant effect on the level of MCHC (g/dL). Treatment of mice with *C. molle* extract at 10, 300 and 1000 mg/kg body weight caused non-significant effect on the levels of RBC ($x10^{12}$/L), HB (g/dL), PCV (%), MCH (pg), MCV (fL) and PLT ($x10^{9}$/L) relative to that of the normal control mice.

Table 4.8: Effects of oral administration of the five aqueous plants extracts to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight on hematological parameters

Plant extract	Dose (mg/kg body weight)	Hematological parameters						
		RBC ($x10^{12}$/L)	HB (g/dL)	PCV (%)	MCH (pg)	MCHC (g/dL)	MCV (fL)	PLT ($x10^{9}$/L)
Amaranthus spinosus	**Control**	**8.05±0.80^{a}**	**11.66±1.57^{a}**	**51.68±10.45^{a}**	**16.08±1.32^{a}**	**23.00±1.98^{a}**	**65.80±6.01^{a}**	**998.0±163.2^{a}**
	10	9.19±1.61^{a}	11.64±1.99^{a}	53.44±4.65^{a}	14.58±1.77^{a}	22.90±2.21^{a}	57.88±7.17^{a}	725.0±417.8^{a}
	300	8.47±1.01^{a}	12.98±1.39^{a}	56.52±6.53^{a}	15.88±0.90^{a}	22.98±2.21^{a}	66.88±6.39^{a}	1095.8±132.7^{a}
	1000	8.93±1.08^{a}	13.24±2.55^{a}	55.08±10.54^{a}	16.04±0.70^{a}	24.92±3.62^{a}	63.08±7.47^{a}	1067.4±113.3^{a}
Cyperus articulatus	**Control**	**7.51±1.50**	**9.42±1.99**	**40.32±10.22**	**12.56±0.10**	**23.54±1.10**	**53.38±4.83**	**439.2±147.6**
	10	7.91±0.70	10.60±0.99	42.22±4.44	13.44±0.86	25.18±1.71	53.36±2.23	393.2±56.7
	300	8.50±0.68	11.28±0.68	48.36±4.61	13.40±1.11	23.50±2.67	57.16±2.46	435.0±159.9
	1000	9.46±2.26	11.46±2.43	50.70±9.08	12.20±1.46	22.78±4.14	54.22±5.30	402.0±115.2
Carissa spinarum	**Control**	**6.33±1.32^{a}**	**9.54±2.11^{a}**	**40.92±2.91^{ab}**	**15.06±0.62^{a}**	**23.28±1.04^{a}**	**64.66±3.05^{a}**	**674.8±193.7^{a}**
	10	**4.18±0.49^{bc}**	**6.90±1.05^{b}**	**31.32±2.11^{bc}**	16.44±0.90^{a}	22.18±1.12^{a}	73.00±4.43^{a}	525.2±104.7^{a}
	300	**3.14±1.16^{c}**	**4.96±1.50^{b}**	**21.86±2.80^{c}**	16.22±1.18^{a}	23.12±1.32^{a}	70.30±8.73^{a}	619.8±143.5^{a}
	1000	6.17±1.29^{ab}	10.48±0.78^{a}	44.34±1.78^{a}	15.88±0.92^{a}	23.72±1.40^{a}	67.68±2.95^{a}	505.0±12.0^{a}
Combretum molle	**Control**	**9.00±1.82^{a}**	**11.42±1.64^{a}**	**64.26±7.94^{a}**	**12.92±1.54^{a}**	**19.14±2.22^{b}**	**67.82±8.80^{a}**	**292.4±42.9^{a}**
	10	9.18±1.61^{a}	11.78±1.24^{a}	53.48±4.26^{a}	13.42±1.75^{a}	**23.52±2.47^{a}**	57.90±6.86^{a}	408.4±91.6^{a}
	300	9.62±2.01^{a}	12.58±2.70^{a}	52.42±9.48^{a}	13.16±1.50^{a}	22.36±2.07^{ab}	59.48±10.53^{a}	475.4±144.8^{a}
	1000	9.53±2.11^{a}	13.82±3.37^{a}	52.44±9.59^{a}	14.44±0.42^{a}	**24.76±2.18^{a}**	58.74±6.03^{a}	582.6±313.4^{a}
Rhynchosia usambarensis	**Control**	**8.83±0.31^{a}**	**12.08±0.82^{a}**	**16.48±1.29^{a}**	**16.48±1.29^{a}**	**24.20±2.87^{a}**	**66.00±7.38^{a}**	**960.0±64.2^{a}**
	10	8.92±0.55^{a}	12.80±1.36^{a}	15.44±1.11^{a}	15.44±1.11^{a}	22.72±2.45^{a}	65.54±5.26^{a}	1064.0±109.0^{a}
	300	9.13±0.81^{a}	11.86±2.40^{a}	15.46±1.59^{a}	15.46±1.59^{a}	23.40±9.09^{a}	62.02±9.57^{a}	1027.6±92.9^{a}
	1000	8.76±1.04^{a}	13.00±2.40^{a}	15.94±1.35^{a}	15.94±1.35^{a}	24.76±3.88^{a}	60.42±8.60^{a}	983.8±53.8^{a}

Results are expressed as mean ± SD of five mice for each dose for each plant extract. Values with the same superscript down the column for each extract dose treatments are not significantly different from each other at $p < 0.05$ by ANOVA followed by Tukey's post hoc test.

4.3.4 Effects of oral administration of the five aqueous plants extracts to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight on white blood cell and differential white blood cell count

The effects of oral administration of the five aqueous plants extracts to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight on white blood cell and differential white blood cell count is reported in Table 4.9. Results indicate that treatment of mice with *A. spinosus* extract at 10 and 1000 mg/kg body weight caused a significant increase in the level of WBC ($x10^9$/L) by 3.0 and 9.9 % compared to that of the normal control mice which was similar to that of the *A. spinosus* extract-treated mice at 300 mg/kg body weight. Further, treatment of mice with *A. spinosus* extract at 10 mg/kg body weight caused a significant reduction in the level of NEU ($x10^9$/L) by 13.95% and at 1000 mg/kg body weight caused a significant increase in the level of NEU ($x10^9$/L) by 13.95% compared to that of the normal control mice which was similar to that of the *A. spinosus* extract-treated mice at 300 mg/kg body weight. In addition, treatment of mice with *A. spinosus* extract at 1000 mg/kg body weight caused a significant increase in the level of LYM ($x10^9$/L) by 2.96 % and at 300 and 1000 mg/kg body weight caused a significant increase in the level of MON ($x10^9$/L) by 4.2 and 35.4 %, respectively, compared to that of the normal control mice which was similar to the *A. spinosus* extract-treated mice at 10 mg/kg body weight dose. Interestingly, treatment of mice with *A. spinosus* extract at the three tested doses demonstrated non-significant effects on the levels of EOS ($x10^9$/L) and BAS ($x10^9$/L) compared to that of the normal control mice.

Treatment of mice with *C. articulatus* extract at 10 and 300 mg/kg body weight caused a significant increase in the level of NEU ($x10^9$/L) by a similar percent of 2.1% which was significantly lower than the increase of 4.8% effected extract-treated mice at 1000 mg/kg body

weight dose compared to that of the normal control mice. Further, treatment of mice with *C. articulatus* extract at 10, 300 and 1000 mg/kg body weight caused a significant increase in the level of LYM ($x10^9$/L) by 3.7, 4.0 and 9.6 % compared to that of the normal control mice. In addition, treatment of mice with *C. articulatus* extract at 300 and 1000 mg/kg body weight caused a significant increase in the level of MON ($x10^9$/L) by a similar percent (0.68 and 1.36 %, respectively) compared to that of the normal control mice which was similar to that of the extract-treated mice at 10 mg/kg body weight dose. Interestingly, treatment of mice with *C. articulatus* extract at 10, 300 and 1000 mg/kg body weight caused a non-significant effect on the levels of WBC ($x10^9$/L), EOS ($x10^9$/L) and BAS ($x10^9$/L) compared to that of the normal control mice.

Treatment of mice with *C. spinarum* extract at 300 and 1000 mg/kg body weight caused a significant increase in WBC ($x10^9$/L, [10 and 10.2 %, respectively]), NEU ($x10^9$/L, [8.2 and 11%, respectively]), and LYM ($x10^9$/L, [9.1 and 10.4%, respectively]) compared to that of the normal control mice which was similar to the extract-treated mice at 10 mg/kg body weight dose. In addition, treatment of mice with *C. spinarum* extract at 10, 300 and 1000 mg/kg body weight caused a significant increase in the level of MON ($x10^9$/L) by 1.3, 9 and 9 % compared to that of the normal control mice. Interestingly, treatment of mice with *C. spinarum* extract at 10, 300 and 1000 mg/kg body weight caused a non-significant effect to the levels of EOS ($x10^9$/L) and BAS ($x10^9$/L) compared to that of the normal control mice.

Treatment of mice with *C. molle* extract at 10, 300 and 1000 mg/kg body weight caused a significant increase in the levels of WBC ($x10^9$/L, [0.2, 1.9 and 2.1 %, respectively]), NEU

($x10^9$/L, [1.9, 5.0 and 5.0 %, respectively]), LYM ($x10^9$/L, [1.6, 1.7 and 2.0 %, respectively]) and MON ($x10^9$/L, [6.7, 11.9 and 14.9 %, respectively]) compared to that of the normal control mice. Interestingly, treatment of mice with *C. moll*e extract at all the three tested doses caused a non-significant effect to the levels of EOS ($x10^9$/L) and BAS ($x10^9$/L) which was comparable to that of the normal control mice.

Treatment of mice with *R. usambarensis* at 10, 300 and 1000 mg/kg body weight caused a significant increase in the level of WBC ($x10^9$/L, [0.2, 7.8 and 8.0 %, respectively]) and MON ($x10^9$/L, [2.1, 9.7 and 12.4%, respectively]) compared to that of the normal control mice. Further, treatment of mice with *R. usambarensis* at 300 and 1000 mg/kg body weight caused a significant increase in the level of LYM ($x10^9$/L, [5.1 and 6.6 %, respectively]) compared to that of the normal control mice which was similar to that of the extract-treated mice at 10 mg/kg body weight dose. Interestingly, treatment of mice with *R. usambarensis* at 10, 300 and 1000 mg/kg body weight caused a non-significant effect to the levels of NEU ($x10^9$/L), EOS ($x10^9$/L) and BAS ($x10^9$/L) which were comparable to that of the normal control mice.

Table 4.9: Effects of oral administration of the five aqueous plants extracts to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight on white blood cell and differential white blood cell count

Plant extracts	Dose (mg/kg body weight)	White blood cell and differential white blood cell count					
		WBC ($x10^9$/L)	NEU ($x10^9$/L)	LYM ($x10^9$/L)	MON ($x10^9$/L)	EOS ($x10^9$/L)	BAS ($x10^9$/L)
Amaranthus spinosus	**Control**	**9.32±0.04^{c}**	**1.29±0.01^{b}**	**6.42±0.02^{b}**	**1.44±0.01^{c}**	**0.09±0.00^{a}**	**0.01±0.00^{a}**
	10	**9.60±1.01^{b}**	**1.11±0.01^{c}**	**6.45±0.02^{b}**	**1.45±0.01^{c}**	0.09±0.00^{a}	0.01±0.00^{a}
	300	**9.23±0.27^{c}**	**1.31±0.02^{b}**	**6.39±0.02^{c}**	**1.50±0.00^{b}**	0.09±0.00^{a}	0.01±0.02^{a}
	1000	**10.24±0.02^{a}**	**1.47±0.01^{a}**	**6.61±0.01^{a}**	**1.95±0.01^{a}**	0.09±0.01^{a}	0.01±0.00^{a}
Cyperus articulatus	**Control**	**9.54±0.01^{a}**	**1.46±0.00^{c}**	**6.45±0.02^{d}**	**1.47±0.01^{b}**	**0.09±0.01^{b}**	**0.01±0.00^{a}**
	10	9.98±0.02^{a}	**1.49±0.01^{b}**	6.69±0.00^{c}	**1.46±0.01^{b}**	**0.10±0.01^{ab}**	0.01±0.00^{a}
	300	10.05±0.02^{a}	**1.49±0.01^{b}**	**6.71±0.01^{b}**	**1.48±0.01^{a}**	**0.10±0.01^{ab}**	0.01±0.00^{a}
	1000	10.32±0.02^{a}	**1.53±0.01^{a}**	**7.07±0.01^{a}**	**1.49±0.01^{a}**	**0.11±0.01^{a}**	0.01±0.00^{a}
Carissa spinarum	**Control**	**9.27±0.01^{c}**	**1.46±0.01^{c}**	**6.16±0.01^{c}**	**1.45±0.01^{c}**	**0.11±0.01^{c}**	**0.02±0.00^{a}**
	10	**9.28±1.11^{c}**	**1.46±0.01^{c}**	**6.16±0.01^{c}**	**1.47±0.01^{b}**	**0.10±0.01^{b}**	0.02±0.00^{a}
	300	**10.20±0.01^{b}**	**1.58±0.01^{b}**	**6.72±0.02^{b}**	**1.58±0.01^{a}**	**0.11±0.01^{c}**	0.01±0.00^{a}
	1000	**10.22±0.02^{a}**	**1.62±0.02^{a}**	**6.80±0.01^{a}**	**1.58±0.01^{a}**	**0.11±0.01^{c}**	0.01±0.00^{a}
Combretum molle	**Control**	**10.01±0.01^{d}**	**1.59±0.01^{c}**	**6.93±0.01^{c}**	**1.34±0.01^{d}**	**0.10±0.01**^{a}	**0.01±0.00^{a}**
	10	10.03±0.01^{c}	**1.62±0.01^{b}**	**7.04±0.01^{b}**	1.43±0.01^{c}	0.10±0.01^{a}	0.01±0.00^{a}
	300	**10.20±0.51^{b}**	**1.67±0.01^{a}**	**7.05±0.01^{b}**	**1.50±0.01^{b}**	0.11±0.01^{a}	0.01±0.00^{a}
	1000	**10.23±0.04^{a}**	**1.67±0.01^{a}**	**7.07±0.01^{a}**	**1.54±0.01^{a}**	0.11±0.01^{a}	0.01±0.00^{a}
Rhynchosia usambarensis	**Control**	**9.39±0.01^{c}**	**1.53±0.01^{a}**	**6.22±0.01^{c}**	**1.45±0.01^{d}**	**0.94±0.01^{a}**	**0.01±0.00^{a}**
	10	**9.41±0.01^{b}**	1.53±0.01^{a}	**6.22±0.01^{c}**	1.48±0.01^{c}	0.09±0.00^{a}	0.01±0.00^{a}
	300	**10.12±0.01^{a}**	1.53±0.01^{a}	**6.54±0.03^{b}**	**1.59±0.01^{b}**	0.08±0.04^{a}	0.01±0.00^{a}
	1000	**10.14±0.01^{a}**	1.54±0.01^{a}	**6.63±0.01^{a}**	**1.63±0.01^{a}**	0.10±0.01^{a}	0.01±0.00^{a}

Results are expressed as mean ± SD of five mice for each dose for each plant extract. Values with the same superscript down the column for each extract dose treatments are not significantly different from each other at $p < 0.05$ by ANOVA followed by Tukey's post hoc test.

4.3.5 Effects of oral administration of the five aqueous plants extracts to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight on biochemical parameters

The effects of oral administration of the five aqueous plants extracts to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight on biochemical parameters are reported in Table 4.10. Results indicate that oral administration of aqueous extracts of *A. spinosus*, *C. articulatus*, *C. molle*, and *R. usambarensis* to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight significantly decreased the levels of GLU (mmol/L) compared to that of the normal control mice in a dose dependent manner. Treatment of mice with aqueous extracts of *A. spinosus*, *C. articulatus*, *C. molle*, and *R. usambarensis* daily for 14 days at 10, 300 and 1000 mg/kg body weight significantly decreased the levels of BUN (mmol/L) compared to that of the normal control mice in a dose independent manner. Treatment of mice with aqueous extracts of *C. spinarum* daily for 14 days at 10, 300 and 1000 mg/kg body weight caused a non-significant effect to the level GLU (mmol/L) and BUN (mmol/L) which was comparable to that of the normal control mice.

Treatment of mice with *A. spinosus* extract at 300 mg/kg body weight dose significantly increased the level of CREAT compared to that of the normal control mice which is similar to the extract-treated mice at 10 mg/kg body weight dose. *A. spinosus* extract-treated mice at 1000 mg/kg body weight dose caused a significantly lower level of CREAT compared to that of the normal control mice. *A. spinosus* extract-treated mice at 10 and 300 mg/kg body weight doses caused a similar effect to the level of CREAT which was significantly higher than that of the extract-treated mice at 1000 mg/kg body weight dose.

Treatment of mice with *A. spinosu*s extract at 10 mg/kg body weight dose caused a non-significant effect to the level T-BIL which was comparable to that of the normal control mice. *A. spinosus* extract-treated mice at 300 and 1000 mg/kg body weight doses caused similar effects to the level of T-BIL which was significantly lower than that of the normal control mice. *A. spinosus* extract-treated mice at 10 and 1000 mg/kg body weight doses caused similar effects to the level of T-BIL. *A. spinosus* extract-treated mice at 300 and 1000 mg/kg body weight doses caused similar effects to the level of T-BIL.

Treatment of mice with *A. spinosus* extract-treated mice at 10, 300 and 1000 mg/kg body weight doses caused similar effects to the level of D-BIL which were comparable to that of the normal control mice. Treatment of mice with *A. spinosus* extract at 10 and 300 mg/kg body weight doses caused similar effects to the activity of ALT which was comparable to that of the normal control mice; these effects were however, significantly lower than that of the extract-treated mice at 1000 mg/kg body weight dose. Treatment of mice with *A. spinosus* extract at 10, 300 and 1000 mg/kg body weight doses caused similar effects to the activity of AST. *A. spinosus* extract-treated mice at 10 mg/kg body weight dose caused a significantly higher activity of AST relative to that of the normal control mice. *A. spinosus* extract-treated mice at 300 and 1000 mg/kg body weight doses caused a non-significant effect to the activity of AST which was comparable to that of the normal control mice.

Treatment of mice with *A. spinosus* extract at 300 and 1000 mg/kg body weight doses caused similar effects to the activity of GGT which was significantly higher than that of the normal control mice. *A. spinosus* extract-treated mice at 10 mg/kg body weight dose caused a non-

significant effect to the activity of GGT which was comparable to that of the normal control mice. *A. spinosus* extract-treated mice at 10, 300 and 1000 mg/kg body weight doses caused similar effects to the activity of GGT.

Treatment of mice with *A. spinosus* extract at 300 mg/kg body weight dose caused a significant increase in the activity of ALP compared to that of the normal control mice. *A. spinosus* extract-treated mice at 10, 300 and 1000 mg/kg body weight doses caused similar effects to the activity of ALP. *A. spinosus* extract-treated mice at 10 and 1000 mg/kg body weight doses caused similar effects to the activity of ALP which was comparable to that of the normal control mice.

Treatment of mice with *C. articulatus* extract at 10 and 300 mg/kg body weight doses caused similar effects to the level of CREAT which was comparable to that of the normal control mice; these effects were however, significantly lower than that of the extract-treated mice at 1000 mg/kg body weight dose.

Treatment of mice with *C. articulatus* extract at 10 mg/kg body weight dose caused a non-significant effect to the level of T-BIL which was comparable to that of the normal control mice. *C. articulatus* extract-treated mice at 1000 mg/kg body weight dose caused a non-significant effect to the level of T-BIL which was comparable to that of the normal control mice. *C. articulatus* extract-treated mice at 300 and 1000 mg/kg body weight doses caused similar effects to the level of T-BIL which was significantly lower than that of the extract-treated mice at 10 mg/kg body weight dose.

Treatment of mice with *C. articulatus* extract at 10 mg/kg body weight dose caused a significant decrease in the level of D-BIL compared to that of the normal control mice. *C. articulatus* extract-treated mice at 300 and 1000 mg/kg body weight doses caused similar effects to the level of D-BIL which was comparable to that of the normal control mice. *C. articulatus* extract-treated mice at 10, 300 and 1000 mg/kg body weight doses caused similar effects to the level of D-BIL.

Treatment of mice with *C. articulatus* extract at 10 and 300 mg/kg body weight doses caused similar effects to the activity of ALT which was comparable to that of the normal control mice; these effects were however, lower than those of the extract-treated mice at 1000 mg/kg body weight dose. *C. articulatus* extract-treated mice at 10, 300 and 1000 mg/kg body weight doses caused similar effects to the activity of AST which was comparable to that of the normal control mice.

Treatment of mice with *C. articulatus* extract at 10 mg/kg body weight dose caused a non-significant effect to the activity of GGT and ALP which was comparable to that of the normal control mice. *C. articulatus* extract-treated mice at 300 mg/kg body weight dose caused a significant increase in the activity of GGT and ALP which was significantly higher than that caused by the extract-treated mice at 1000 mg/kg body weight dose. This was however significantly higher than that of the normal control mice.

Treatment of mice with *C. spinarum* extract at 10, 300 and 1000 mg/kg body weight doses caused non-significant effects to the level of CREAT, D-BIL, ALT, AST, GGT and ALP which were comparable to that of the normal control mice. *C. spinarum* extract-treated mice at 10

mg/kg body weight dose caused a significant increase to the level of T-BIL compared to that of the normal control mice. This was, however, comparable to that of the extract-treated mice at 300 mg/kg body weight dose. *C. spinarum* extract-treated mice at 300 and 1000 mg/kg body weight doses caused similar effects to the level of T-BIL which was comparable to that of the normal control mice. *C. spinarum* extract-treated mice at 300 and 1000 mg/kg body weight doses caused similar effects to the level of T-BIL which was significantly lower than that of the extract-treated mice at 10 mg/kg body weight dose.

Treatment of mice with *C. molle* extract at 10 and 300 mg/kg body weight doses caused similar effects to the levels of CREAT and the activity of ALT which were comparable to that of the normal control mice. *C. molle* extract-treated mice at 1000 mg/kg body weight doses caused a significant decrease in the level of CREAT and a significant increase in the activity of ALT compared to that of the normal control mice. *C. molle* extract-treated mice 10 and 300 mg/kg body weight doses caused similar effects to the level of CREAT and the activity of ALT which was significantly higher and lower, respectively, than that of the extract-treated mice at 1000 mg/kg body weight dose.

Treatment of mice with *C. molle* extract at 10 mg/kg body weight dose caused a non-significant effect to the level of T-BIL and D-BIL which was comparable to that of the normal control mice. *C. molle* extract-treated mice at 300 and 1000 mg/kg body weight doses caused similar effects to the level of T-BIL and D-BIL which were comparable to that of the normal control mice. *C. molle* extract-treated mice at 300 and 1000 mg/kg body weight doses caused similar effects to

the level of T-BIL and D-BIL which were significantly lower than those of the extract-treated mice at 10 mg/kg body weight dose.

Treatment of mice with *C. molle* extract at 10 and 1000 mg/kg body weight doses caused similar effects to the activity of AT, GGT and ALP which were comparable to that of the normal control mice. *C. molle* extract-treated mice at 300 mg/kg body weight caused a significant decrease in the activity of AST and a significant increase in the activity of GGT and ALP, respectively, compared to that of the normal control mice. *C. molle* extract-treated mice at 10 and 1000 mg/kg body weight doses caused similar effects to the activity of GGT and ALP which were significantly higher and lower, respectively, compared to that of the extract-treated mice at 300 mg/kg body weight dose. *C. molle* extract-treated mice at 1000 mg/kg body weight dose caused a significantly higher increase to the activity of GGT than that of the extract-treated mice at 300 mg/kg body weight dose which was significantly than that of the extract-treated mice at 10 mg/kg body weight dose.

Treatment of mice with *R. usambarensis* extract at 10 mg/kg body weight caused a non-significant effect to the level of CREAT which was comparable to that of the normal control mice. *R. usambarensis* extract-treated mice at 10 and 300 mg/kg body weight doses caused similar effects to the level of CREAT which was significantly higher than that of the extract-treated mice at 1000 mg/kg body weight dose. *R. usambarensis* extract-treated mice at 1000 mg/kg body weight dose caused a non-significant effect to the level of CREAT which was comparable to that of the normal control mice.

Treatment of mice with *R. usambarensis* extract at 10 mg/kg body weight caused a non-significant effect to the level of T-BIL which was comparable to that of the normal control mice. *R. usambarensis* extract-treated mice at 300 and 1000 mg/kg body weight doses caused similar effects to the level of T-BIL which was significantly lower than that of the normal control mice. *R. usambarensis* extract-treated mice at 300 and 1000 mg/kg body weight caused similar effects to the level of T-BIL which was significantly lower than that of the extract-treated mice at 10 mg/kg body weight dose. *R. usambarensis* extract-treated mice at 10, 300 and 1000 mg/kg body weight doses caused non-significant effects to the level of D-BIL and the activity of AST which were comparable to that of the normal control mice.

Treatment of mice with *R. usambarensis* extract at 10 and 300 mg/kg body weight doses caused similar effects to the activity of ALT which was comparable to that of the normal control mice. *R. usambarensis* extract-treated mice at 10 and 300 mg/kg body weight doses caused similar effects to the activity of ALT. *R. usambarensis* extract-treated mice at 1000 mg/kg body weight dose caused a significant increase in the activity of ALT compared to that of the normal control mice.

Treatment of mice with *R. usambarensis* extract at 10 mg/kg body weight dose caused a non-significant effect to the activity of GGT which was comparable to that of the normal control mice. *R. usambarensis* extract-treated mice at 300 mg/kg body weight dose caused a significant increase in the activity of GGT comparable to that of the normal control mice. *R. usambarensis* extract-treated mice at 300 mg/kg body weight dose caused a significantly higher increase in the activity of GGT compared to that of the extract-treated mice at 10 mg/kg body weight dose. *R.*

usambarensis extract-treated mice at 10 and 1000 mg/kg body weight doses caused similar effects to the activity of GGT. *R. usambarensis* extract-treated mice caused a similar effect to the activity of GGT.

Treatment of mice with *R. usambarensis* extract at 10 and 1000 mg/kg body weight caused similar effects to the activity of ALP which were comparable to that of the normal control mice. *R. usambarensis* extract-treated mice at 300 mg/kg body weight dose caused a significant increase in the activity of ALP compared to that of the normal control mice. *R. usambarensis* extract-treated 10 and 300 mg/kg body weight doses caused similar effects to the activity of ALP which was significantly higher than that of the extract-treated mice at 1000 mg/kg body weight dose.

Table 4.10: Effects of oral administration of the five aqueous plants extracts to mice daily for 14 days at 10, 300 and 1000 mg/kg body weight on biochemical parameters

Extracts	Analytes concentrations					Enzyme activities			
***Amaranthus spinosus* (mg/kg body weight)**									
	GLU (mmol/L)	BUN (mmol/L)	CREAT (μmol/L)	T-BIL (μmol/L)	D-BIL (μmol/L)	ALT (U/L)	AST (U/L)	GGT (U/L)	ALP (U/L)
Control	**4.77±0.30^{a}**	**5.34±0.94^{a}**	**7.68±2.81^{bc}**	**17.52±1.80^{a}**	**5.16±0.54^{a}**	**38.40±2.52^{b}**	**10.06±3.29^{b}**	**3.58±0.57^{b}**	**7.40±2.07^{b}**
10	**3.58±0.21^{b}**	**3.09±0.45^{b}**	9.54±1.49^{ab}	16.02±2.97^{ab}	5.08±0.59^{a}	**39.60±1.14^{b}**	**18.92±4.80^{a}**	6.90±2.83^{ab}	8.00±1.58^{ab}
300	**3.31±0.12^{bc}**	**2.85±0.42^{b}**	**12.42±2.10^{a}**	**10.14±2.29^{c}**	4.54±0.60^{a}	**42.80±3.96^{b}**	13.60±3.05^{ab}	**9.14±2.47^{a}**	**11.72±3.56^{a}**
1000	**2.83±0.43^{c}**	**2.92±0.14^{b}**	4.98±1.26^{c}	**12.76±2.72^{bc}**	4.34±0.54^{a}	**61.40±5.04^{a}**	16.70±4.75^{ab}	**10.14±2.3^{a}**	7.60±1.14^{ab}
***Cyperus articulatus* (mg/kg body weight)**									
Control	**4.55±0.51^{a}**	**6.20±0.84^{a}**	**9.50±0.81^{a}**	**16.72±2.21^{ab}**	**5.98±1.50^{a}**	**37.00±2.74^{b}**	**20.20±6.40^{b}**	**4.30±0.58^{c}**	**8.20±1.92^{b}**
10	**3.42±0.21^{b}**	**3.69±0.55^{b}**	**11.18±1.03^{a}**	17.42±1.20^{a}	**3.96±0.24^{b}**	**40.20±2.17^{b}**	**35.60±12.84^{b}**	**4.72±0.50^{c}**	**8.40±0.89^{b}**
300	**3.60±0.25^{b}**	**2.90±0.29^{b}**	**10.28±0.79^{a}**	**13.36±1.39^{c}**	4.78±0.59^{ab}	**41.40±1.95^{b}**	**13.70±3.78^{b}**	**10.20±1.39^{a}**	**12.48±3.63^{a}**
1000	**2.75±0.24^{c}**	**2.84±0.15^{b}**	**6.60±1.15^{b}**	13.90±1.50^{bc}	4.70±0.70^{ab}	**54.00±10.54^{a}**	**15.02±2.92^{b}**	**7.70±2.03^{b}**	**7.40±1.14^{b}**
***Carissa spinarum* (mg/kg body weight)**									
Control	**4.62±1.07^{a}**	**7.06±1.13^{a}**	**18.60±2.80^{a}**	**7.42±1.46^{b}**	**5.72±1.47^{a}**	**34.20±4.68^{a}**	**33.64±8.70^{a}**	**1.60±0.74^{a}**	**4.60±1.44^{a}**
10	**4.12±0.73^{a}**	**4.30±1.23^{a}**	**42.40±5.26^{a}**	**13.30±1.93^{a}**	**5.32±1.02^{a}**	**17.40±3.77^{b}**	**19.80±7.14^{a}**	**2.20±1.32^{a}**	**3.60±1.30^{a}**
300	**4.86±0.93^{a}**	**6.06±1.41^{a}**	**28.60±3.32^{a}**	**7.10±1.37^{b}**	**4.16±1.12^{a}**	**26.20±3.96^{a}**	**21.26±7.13^{a}**	**1.20±1.10^{a}**	**3.60±1.44^{a}**
1000	**4.82±1.36^{a}**	**10.50±2.85^{a}**	**44.60±4.39^{a}**	8.28±1.94^{ab}	**4.34±1.41^{a}**	**26.20±4.79^{a}**	**29.22±10.62^{a}**	**1.20±0.67^{a}**	**3.40±1.70^{a}**
***Combretum molle* (mg/kg body weight)**									
Control	**5.18±0.51^{a}**	**5.66±0.59^{a}**	**8.58±1.10^{a}**	**16.1±2.14^{ab}**	**4.58±0.15^{ab}**	**37.60±2.07^{b}**	**20.80±4.55^{a}**	**4.60±0.81^{c}**	**7.20±1.64^{b}**
10	**3.42±0.24^{b}**	**3.43±0.40^{b}**	**10.02±1.95^{a}**	20.34±3.12^{a}	6.66±2.25^{a}	**41.00±1.87^{b}**	**17.42±2.89^{a}**	**3.94±0.70^{c}**	**8.00±1.58^{b}**
300	**3.52±0.16^{b}**	**3.11±0.52^{b}**	**9.92±0.55^{a}**	**14.36±3.90^{b}**	**4.38±0.37^{b}**	**42.00±2.24^{b}**	**10.00±2.16^{b}**	**9.22±1.29^{b}**	**12.08±1.83^{a}**
1000	**2.90±0.36^{c}**	**3.01±0.89^{b}**	**6.00±0.82^{b}**	**11.32±0.97^{b}**	**4.32±0.35^{b}**	**58.00±5.15^{a}**	**21.72±2.02^{a}**	**11.16±1.08^{a}**	**8.20±0.84^{b}**
***Rhynchosia usambarensis* (mg/kg body weight)**									
Control	**5.28±0.48^{a}**	**5.68±1.56^{a}**	**6.24±1.14^{bc}**	**18.23±9.57^{a}**	**6.86±2.10^{a}**	**35.60±3.21^{b}**	**8.28±4.05^{a}**	**2.96±0.68^{c}**	**6.40±2.30^{b}**
10	**3.57±0.39^{b}**	**3.62±0.88^{b}**	**9.60±3.33^{ab}**	20.30±4.70^{ab}	**7.28±2.31^{a}**	38.80±1.92^{ab}	**12.28±4.34^{a}**	4.16±0.76^{bc}	7.80±1.92^{ab}
300	**3.50±0.24^{bc}**	**2.76±0.44^{b}**	**10.34±1.61^{a}**	**12.18±2.44^{c}**	**4.38±0.58^{a}**	42.60±5.59^{ab}	**10.64±4.33^{a}**	**8.16±2.50^{a}**	**11.14±3.47^{a}**
1000	**2.83±0.40^{c}**	**2.55±0.79^{b}**	**5.06±1.69^{c}**	13.60±5.28^{bc}	**5.20±2.34^{a}**	**52.80±14.31^{a}**	**7.62±1.64^{a}**	**7.56±3.18^{ab}**	**6.20±1.30^{b}**

Results are expressed as mean ± SD of five mice for each dose for each plant extract. Values with the same superscript down the column for each extract dose treatments are not significantly different from each other at $p < 0.05$ by ANOVA followed by Tukey's post hoc test.

4.4 Qualitative phytochemical screening of the five aqueous plants extracts

Preliminary qualitative phytochemical screening of the five aqueous extracts revealed the presence of alkaloids in *A. spinosus, C. articulatus*, and *C. molle* but absence in *C. spinarum* and *R. usambarensis*; saponins in *A. spinosus, C. articulatus,* and *C. spinarum* but absence in *C. molle* and *R. usambarensis;* tannins in *A. spinosus*, *C. articulatus*, *C. spinarum*, *C. molle*, and *R. usambarensis*; cardiac glycosides in *C. spinarum* and *R. usambarensis* but absence in *A. spinosus, C. articulatus* and *C. molle*; steroids in *R. usambarensis* but absence in *A. spinosus, C. articulatus, C. molle* and *C. spinarum*; terpenoids in *A. spinosus*, *C. articulatus*, *C. spinarum*, and *C. molle* but absence in *R. usambarensis*; triterpenoids in *C. articulatus* but absence in *A. spinosus, C. spinarum, C. molle*, and *R. usambarensis*; flavonoids in *R. usambarensis* but absence in *A. spinosus*, *C. articulatus*, *C. spinarum*, and *C. molle*; flavones in *C. spinarum* and *C. molle* but absence in *A. spinosus, C. articulatus* and *R. usambarensis*; and phenols in *C. articulatus, C. spinarum, C. molle*, and *R. usambarensis* but absence in *A. spinosus* (Table 4.11).

Table 4.11: Phytochemical profile of the five aqueous plants extracts

Phytochemicals	***A. spinosus***	***C. articulatus***	***C. spinarum***	***C. molle***	***R. usambarensis***
Alkaloids	+	+	-	+	-
Tannins	+	+	+	+	+
Cardiac glycosides	-	-	+	-	+
Steroids	-	-	-	-	+
Triterpenoids	-	+	-	-	-
Flavonoids	-	-	-	-	+
Terpenoids	+	+	+	+	-
Phenols	-	+	+	+	+
Flavones	-	-	+	+	-
Saponins	+	+	+	-	-

KEY: + Presence; - Negative results

4.5 Mineral elements composition of the five aqueous plants extracts

In this study, the concentrations of seventeen elements were determined in aqueous extracts of *A. spinosus*, *C. articulatus*, *C. spinarum*, *C. molle* and *R. usambarensis*. The mean (n = 3) concentrations of various mineral elements in the five aqueous plants extracts including K, Ca, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Br, Rb, Sr, Hg and Pb are shown in Table 4.12, 4.13, 4.14, 4.15 and 4.16, respectively. All the measured mineral elements were present in all the five plants extracts at varying concentrations. However, V (*C. spinarum*), Cr (*C. spinarum*, *C. molle*, *R. usambarensis*), Ni (*C. molle*, *R. usambarensis*), Se (*A. spinosus*, *C. articulatus*, *C. spinarum*, *C. molle*, *R. usambarensis*), Hg (*A. spinosus*, *C. articulatus*, *C. spinarum*, *C. molle*, *R. usambarensis*), and Pb (*A. spinosus*) were below detection levels in the indicated aqueous plants extracts. The quantities of twelve of the mineral elements orally administered to mice daily for 14 days for all five aqueous plants extracts at the three tested dose levels of this study were found to be lower than their recommended daily allowance (RDA). Further, the quantities of V (*R. usambarensis*, *A. spinosus*, *C. molle*, *C. articulatus*), Mn (*R. usambarensis*, *C. molle*, *C. articulatus*), Cr (*A. spinosus*, *C. articulatus*), Fe (*A. spinosus*, *C. articulatus*), and Pb (*C. spinarum*, *C. molle*) were above the recommended daily allowances (in the indicated aqueous plants extracts).

Table 4.12: Mineral element composition and their quantities in 0.18, 5.4 and 18 mg of aqueous extracts of *A. spinosus* administered to each mouse per day for 14 days

Mineral element	Concentration in µg/g	10 (mg/kg body weight)	300 (mg/kg body weight)	1000 (mg/kg body weight)	RDA (mg/20g)
K	6774.78±162.6	$1.21946x10^{-3}$	$3.65838x10^{-2}$	$1.21946x10^{-1}$	$6.15385x10^{-1}$
Ca	331.63±8.38	$5.96934x10^{-5}$	$1.79080x10^{-3}$	$5.96934x10^{-3}$	$3.07692x10^{-1}$
Ti	12.54±0.40	$2.2572x10^{-6}$	$6.7716x10^{-5}$	$2.2572x10^{-4}$	
V	2.08±0.12	$3.744x10^{-7}$	$1.1232x10^{-5}$	**$3.744x10^{-5}$**	$9.23076x10^{-6}$
Cr	0.62±0.06	$1.116x10^{-7}$	$3.348x10^{-6}$	**$1.116x10^{-5}$**	$1.07692x10^{-5}$
Mn	23.31±0.61	$4.1958x10^{-6}$	$1.25874x10^{-4}$	$4.1958x10^{-4}$	$7.07692x10^{-4}$
Fe	171.47±4.22	$3.08646x10^{-5}$	$9.25938x10^{-4}$	**$3.08646x10^{-3}$**	$2.46154x10^{-3}$
Ni	0.20±0.02	$3.6x10^{-8}$	$1.08x10^{-6}$	$3.6x10^{-6}$	$1.8462x10^{-5}$
Cu	1.15±0.05	$2.07x10^{-7}$	$6.21x10^{-6}$	$2.07x10^{-5}$	$4.6154x10^{-4}$
Zn	8.75±0.24	$1.575x10^{-6}$	$4.725x10^{-5}$	$1.575x10^{-4}$	$3.38462x10^{-3}$
As	0.10±0.01	$1.8x10^{-8}$	$5.4x10^{-7}$	$1.8x10^{-6}$	$1.84615x10^{-5}$
Se	< 0.04	$< 7.2x10^{-9}$	$< 2.16x10^{-7}$	$< 7.2x10^{-7}$	$1.69231x10^{-5}$
Br	4.66±0.13	$8.388x10^{-7}$	$2.5164x10^{-5}$	$8.388x10^{-5}$	$2.46154x10^{-3}$
Rb	6.80±0.18	$1.224x10^{-6}$	$3.672x10^{-5}$	$1.224x10^{-4}$	$1.53846x10^{-3}$
Sr	10.92±0.29	$1.9656x10^{-6}$	$5.8969x10^{-5}$	$1.9656x10^{-4}$	
Hg	< 0.10	$< 1.8x10^{-8}$	$< 5.4x10^{-7}$	$< 1.8x10^{-7}$	
Pb	< 0.08	$< 1.44x10^{-8}$	$< 4.32x10^{-7}$	$< 1.44x10^{-6}$	$3.07692x10^{-5}$

Edori and Marcus (2017) and Strain and Cashman (2009) were the sources of RDA mineral element values. Amount of mineral administered in each mouse based on the test dose is in mg. The mineral elements with RDAs or AI included: selenium [55µg/day], manganese [2.3mg/day], copper [1.5mg/day], bromine [2-8mg/day], arsenic [12-60µg/day], nickel [70-260µg], chromium [35µg], vanadium [10-30µg], rubidium [1-5mg], lead [15-100µg], calcium [1000mg], potassium [2000mg], iron [8mg], and zinc [11mg].

Table 4.13: Mineral element composition and their quantities in 0.18, 5.4 and 18 mg of aqueous extracts of *C. articulatus* administered to each mouse per day for 14 days

Mineral element	Concentration in µg/g	10 (mg/kg body weight)	300 (mg/kg body weight)	1000 (mg/kg body weight)	RDA (mg/20g)
K	8148.32±280.77	$1.46667x10^{-3}$	$4.39992x10^{-2}$	$1.4667x10^{-1}$	$6.15385x10^{-1}$
Ca	95.63±3.70	$1.72134x10^{-5}$	$5.16402x10^{-4}$	$1.72134x10^{-3}$	$3.07692x10^{-1}$
Ti	4.36±0.27	$7.848x10^{-7}$	$2.3544x10^{-5}$	$7.848x10^{-5}$	
V	1.56±0.15	$2.808x10^{-7}$	$8.424x10^{-6}$	**$2.808x10^{-5}$**	$9.23076x10^{-6}$
Cr	0.67±0.01	$1.206x10^{-7}$	$3.618x10^{-6}$	**$1.206x10^{-5}$**	$1.07692x10^{-5}$
Mn	47.49±1.76	$8.5482x10^{-6}$	$2.56446x10^{-4}$	**$8.5482x10^{-4}$**	$7.07692x10^{-4}$
Fe	194.98±6.85	$3.50964x10^{-5}$	$1.05289x10^{-3}$	**$3.50964x10^{-3}$**	$2.46154x10^{-3}$
Ni	0.73±0.05	$1.314x10^{-7}$	$3.942x10^{-6}$	$1.314x10^{-5}$	$1.8462x10^{-5}$
Cu	2.73±0.12	$4.914x10^{-7}$	$1.4742x10^{-5}$	$4.914x10^{-5}$	$4.6154x10^{-4}$
Zn	13.75±0.52	$2.475x10^{-6}$	$7.425x10^{-5}$	$2.475x10^{-4}$	$3.38462x10^{-3}$
As	0.41±0.03	$7.38x10^{-8}$	$2.214x10^{-6}$	$7.38x10^{-6}$	$1.84615x10^{-5}$
Se	< 0.04	$< 7.2x10^{-9}$	$< 2.16x10^{-7}$	$< 7.2x10^{-7}$	$1.69231x10^{-5}$
Br	14.11±0.15	$2.5398x10^{-6}$	$7.6194x10^{-5}$	$2.5398x10^{-4}$	$2.46154x10^{-3}$
Rb	6.18±0.24	$1.1124x10^{-6}$	$3.3372x10^{-5}$	$1.1124x10^{-4}$	$1.53846x10^{-3}$
Sr	2.25±0.10	$4.05x10^{-7}$	$1.215x10^{-5}$	$4.05x10^{-5}$	
Hg	< 0.10	$< 1.8x10^{-8}$	$< 5.4x10^{-7}$	$< 1.8x10^{-7}$	
Pb	1.08±0.06	$1.944x10^{-7}$	$5.832x10^{-6}$	$1.944x10^{-5}$	$3.07692x10^{-5}$

Edori and Marcus (2017) and Strain and Cashman (2009) were the sources of RDA mineral element values. Amount of mineral administered in each mouse based on the test dose is in mg. The mineral elements with RDAs or AI included: selenium [55µg/day], manganese [2.3mg/day], copper [1.5mg/day], bromine [2-8mg/day], arsenic [12-60µg/day], nickel [70-260µg], chromium [35µg], vanadium [10-30µg], rubidium [1-5mg], lead [15-100µg], calcium [1000mg], potassium [2000mg], iron [8mg], and zinc [11mg].

Table 4.14: Mineral element composition and their quantities in 0.18, 5.4 and 18 mg of aqueous extracts of *C. spinarum* administered to each mouse per day for 14 days

Mineral element	Concentration in µg/g	10 (mg/kg body weight)	300 (mg/kg body weight)	1000 (mg/kg body weight)	RDA (mg/20g)
K	3963.45±89.33	$7.13421x10^{-4}$	$2.14026x10^{-2}$	$7.13421x10^{-2}$	$6.15385x10^{-1}$
Ca	459.10±1079	$8.2638x10^{-5}$	$2.47914x10^{-3}$	$8.2638x10^{-3}$	$3.07692x10^{-1}$
Ti	0.58±0.01	$1.044x10^{-7}$	$3.132x10^{-6}$	$1.044x10^{-5}$	
V	< 0.23	$< 4.14x10^{-8}$	$< 1.242x10^{-6}$	$< 4.14x10^{-6}$	$9.23076x10^{-6}$
Cr	< 0.15	$< 2.7x10^{-8}$	$< 8.1x10^{-7}$	$< 2.7x10^{-6}$	$1.07692x10^{-5}$
Mn	16.75±0.45	$3.015x10^{-6}$	$9.045x10^{-5}$	$3.015x10^{-4}$	$7.07692x10^{-4}$
Fe	43.70±0.02	$7.866x10^{-6}$	**$2.3598x10^{-4}$**	**$7.866x10^{-4}$**	$2.46154x10^{-3}$
Ni	0.18±0.01	$3.24x10^{-8}$	$9.72x10^{-7}$	$3.24x10^{-6}$	$1.8462x10^{-5}$
Cu	1.82±0.06	$3.276x10^{-7}$	$9.828x10^{-6}$	$3.276x10^{-5}$	$4.6154x10^{-4}$
Zn	5.55±0.15	$9.99x10^{-7}$	$2.997x10^{-5}$	$9.99x10^{-5}$	$3.38462x10^{-3}$
As	0.10±0.04	$1.8x10^{-8}$	$5.4x10^{-7}$	$1.8x10^{-7}$	$1.84615x10^{-5}$
Se	< 0.04	$< 7.2x10^{-9}$	$< 2.16x10^{-7}$	$< 7.2x10^{-7}$	$1.69231x10^{-5}$
Br	5.28±0.14	$9.504x10^{-7}$	$2.8512x10^{-5}$	$9.504x10^{-5}$	$2.46154x10^{-3}$
Rb	6.30±0.16	$1.134x10^{-6}$	$3.402x10^{-5}$	$1.134x10^{-4}$	$1.53846x10^{-3}$
Sr	3.43±0.10	$6.174x10^{-7}$	$1.8522x10^{-5}$	$6.174x10^{-5}$	
Hg	< 0.10	$< 1.8x10^{-8}$	$< 5.4x10^{-7}$	$< 1.8x10^{-7}$	
Pb	6.12±0.17	$1.1016x10^{-6}$	**$3.3048x10^{-5}$**	**$1.1016x10^{-4}$**	$3.07692x10^{-5}$

Edori and Marcus (2017) and Strain and Cashman (2009) were the sources of RDA mineral element values. Amount of mineral administered in each mouse based on the test dose is in mg. The mineral elements with RDAs or AI included: selenium [55µg/day], manganese [2.3mg/day], copper [1.5mg/day], bromine [2-8mg/day], arsenic [12-60µg/day], nickel [70-260µg], chromium [35µg], vanadium [10-30µg], rubidium [1-5mg], lead [15-100µg], calcium [1000mg], potassium [2000mg], iron [8mg], and zinc [11mg].

Table 4.15: Mineral element composition and their quantities in 0.18, 5.4 and 18 mg of aqueous extracts of *C. molle* administered to each mouse per day for 14 days

Mineral element	Concentration in µg/g	10 (mg/kg body weight)	300 (mg/kg body weight)	1000 (mg/kg body weight)	RDA (mg/20g)
K	3710.30±112.17	$6.67854x10^{-4}$	$2.00356x10^{-2}$	$6.67854x10^{-2}$	$6.15385x10^{-1}$
Ca	367.30±11.78	$6.6114x10^{-5}$	$1.98342x10^{-3}$	$6.6114x10^{-3}$	$3.07692x10^{-1}$
Ti	0.73±0.13	$1.314x10^{-7}$	$3.942x10^{-6}$	$1.314x10^{-5}$	
V	1.20±0.13	$2.16x10^{-7}$	$6.48x10^{-6}$	**$2.16x10^{-5}$**	$9.23076x10^{-6}$
Cr	< 0.15	$< 2.7x10^{-8}$	$< 8.1x10^{-7}$	$< 2.7x10^{-6}$	$1.07692x10^{-5}$
Mn	45.53±1.50	$8.1954x10^{-6}$	$2.45862x10^{-4}$	**$8.1954x10^{-4}$**	$7.07692x10^{-4}$
Fe	17.19±0.60	$3.0942x10^{-6}$	$9.2826x10^{-5}$	**$3.0942x10^{-4}$**	$2.46154x10^{-3}$
Ni	< 0.08	$< 1.44x10^{-8}$	$< 4.32x10^{-7}$	$< 1.44x10^{-6}$	$1.8462x10^{-5}$
Cu	2.26±0.10	$4.068x10^{-7}$	$1.2204x10^{-5}$	$4.068x10^{-5}$	$4.6154x10^{-4}$
Zn	32.82±1.05	$5.9076x10^{-6}$	$1.77228x10^{-4}$	$5.9076x10^{-4}$	$3.38462x10^{-3}$
As	0.93±0.05	$1.674x10^{-7}$	$5.022x10^{-6}$	$1.674x10^{-5}$	$1.84615x10^{-5}$
Se	< 0.04	$< 7.2x10^{-9}$	$< 2.16x10^{-7}$	$< 7.2x10^{-7}$	$1.69231x10^{-5}$
Br	5.34±0.19	$9.612x10^{-7}$	$2.8836x10^{-5}$	$9.612x10^{-5}$	$2.46154x10^{-3}$
Rb	5.43±0.19	$9.774x10^{-7}$	$2.9322x10^{-5}$	$9.774x10^{-5}$	$1.53846x10^{-3}$
Sr	6.04±0.21	$1.0872x10^{-6}$	$3.2616x10^{-5}$	$1.0872x10^{-4}$	
Hg	< 0.10	$< 1.8x10^{-8}$	$< 5.4x10^{-7}$	$< 1.8x10^{-7}$	
Pb	5.12±0.19	$9.216x10^{-7}$	$2.7648x10^{-5}$	**$9.216x10^{-5}$**	$3.07692x10^{-5}$

Edori and Marcus (2017) and Strain and Cashman (2009) were the sources of RDA mineral element values. Amount of mineral administered in each mouse based on the test dose is in mg. The mineral elements with RDAs or AI included: selenium [55µg/day], manganese [2.3mg/day], copper [1.5mg/day], bromine [2-8mg/day], arsenic [12-60µg/day], nickel [70-260µg], chromium [35µg], vanadium [10-30µg], rubidium [1-5mg], lead [15-100µg], calcium [1000mg], potassium [2000mg], iron [8mg], and zinc [11mg].

Table 4.16: Mineral element composition and their quantities in 0.18, 5.4 and 18 mg of aqueous extracts of *R. usambarensis* administered to each mouse per day for 14 days

Mineral	Concentration in µg/g	10 (mg/kg body weight)	300 (mg/kg body weight)	1000 (mg/kg body weight)	RDA (mg/20g)
K	4138.64	7.44955×10^{-4}	2.23487×10^{-2}	7.44955×10^{-2}	6.15385×10^{-1}
Ca	827.38±34.78	1.48928×10^{-4}	4.46785×10^{-3}	1.48928×10^{-2}	3.07692×10^{-1}
Ti	1.35±0.15	2.43×10^{-7}	7.29×10^{-6}	2.43×10^{-5}	
V	1.17±0.19	2.106×10^{-7}	6.318×10^{-6}	**2.106×10^{-5}**	9.23076×10^{-6}
Cr	< 0.15	$< 2.7 \times 10^{-8}$	$< 8.1 \times 10^{-7}$	$< 2.7 \times 10^{-6}$	1.07692×10^{-5}
Mn	48.59±2.18	8.7462×10^{-6}	2.62386×10^{-4}	**8.7462×10^{-4}**	7.07692×10^{-4}
Fe	18.64±0.88	3.3552×10^{-6}	1.00656×10^{-4}	3.3552×10^{-4}	2.46154×10^{-3}
Ni	< 0.08	$< 1.44 \times 10^{-8}$	$< 4.32 \times 10^{-7}$	$< 1.44 \times 10^{-6}$	1.8462×10^{-5}
Cu	1.34±0.10	2.412×10^{-7}	7.236×10^{-6}	2.412×10^{-5}	4.6154×10^{-4}
Zn	4.34±0.23	7.812×10^{-7}	2.3436×10^{-5}	7.812×10^{-5}	3.38462×10^{-3}
As	0.19±0.03	3.42×10^{-8}	1.026×10^{-6}	3.42×10^{-6}	1.84615×10^{-5}
Se	< 0.04	$< 7.2 \times 10^{-9}$	$< 2.16 \times 10^{-7}$	$< 7.2 \times 10^{-7}$	1.69231×10^{-5}
Br	7.35±0.34	1.323×10^{-6}	3.969×10^{-5}	1.323×10^{-4}	2.46154×10^{-3}
Rb	6.23±0.29	1.1214×10^{-6}	3.3642×10^{-5}	1.1214×10^{-4}	1.53846×10^{-3}
Sr	7.24±0.34	1.3032×10^{-6}	3.9096×10^{-5}	1.3032×10^{-4}	
Hg	< 0.10	$< 1.8 \times 10^{-8}$	$< 5.4 \times 10^{-7}$	$< 1.8 \times 10^{-6}$	
Pb	0.31±0.04	5.58×10^{-8}	1.674×10^{-6}	5.58×10^{-6}	3.07692×10^{-5}

Edori and Marcus (2017) and Strain and Cashman (2009) were the sources of RDA mineral element values. Amount of mineral administered in each mouse based on the test dose is in mg. The mineral elements with RDAs or AI included: selenium [55µg/day], manganese [2.3mg/day], copper [1.5mg/day], bromine [2-8mg/day], arsenic [12-60µg/day], nickel [70-260µg], chromium [35µg], vanadium [10-30µg], rubidium [1-5mg], lead [15-100µg], calcium [1000mg], potassium [2000mg], iron [8mg], and zinc [11mg].

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 DISCUSSION

Snakebite is a major public health hazard that leads to high mortality rate worldwide. World Health Organization (WHO) estimates that 2.5 million snakebites occur worldwide each year and out of these, 125,000 are fatal (Ramos and Ho, 2015). Snake antivenoms are currently the only validated antidotes for snake envenomation, but they have a number of limitations which include hypersensitivity reactions among some patients and inefficient supply system leading to their unavailability in rural areas. Although use of plants against the effects of snake bites has long been recognized, more scientific attention has only been given to this area of research in the last 20 years (Rita *et al*., 2011). The present study examined antivenom potential, phytochemical composition, mineral elements composition and acute and sub-acute toxicity effects of aqueous extracts of *Amaranthus spinosus*, *Cyperus articulatus*, *Carissa spinarum*, *Combretum molle* and *Rhynchosia usambarensis*. The most important step in determining antivenom potential of plant extracts is the pre-clinical testing using *in vivo* and *in vitro* methods to assess their neutralizing potential against a wide range of venom effects (Meenatchisundaram *et al*., 2008).

The only validated protocol for assessing venom toxicity and antivenom neutralizing potency by both manufacturers and regulatory authorities is the test for determining venom lethality (LD_{50}) and antivenom neutralizing capacity (ED_{50}) (WHO, 2010a). In this study, therefore, *in vivo* and *in vitro* pharmacological tests for venom lethality, procoagulant activity and phospholipase activity (PLA_2) caused by *Naja subfulva* venom were investigated. The design for the conventional antivenoms rodent ED_{50} tests requires premixing of the venom with the test

antivenom before intravenous injection into the animal (Sells, 2003). This however, does not replicate the clinical situation where the events occur at different times, that is, envenomation followed by antivenom therapy. Following this protocol, neutralization studies on the plant extracts showed that the aqueous extracts of *Amaranthus spinosus*, *Cyperus articulatus*, *Carissa spinarum*, *Combretum molle* and *Rhynchosia usambarensis* exhibited varying abilities to neutralize the lethality induced by *N. subfulva* venom in a dose dependent manner. Since the snake venom is mainly made up of proteinaceous compounds (approximately 90-95%) mainly enzymes, the most likely mechanism for the plant extracts' ability to neutralize *N. subfulva* venom could be due to the ability of the extracts triterpenoids, terpenoids, polyphenolic, tannins and tannin like substances to bind venom proteins resulting in inhibition and precipitation of the venom proteins (Pithayanukul *et al*., 2005).

Naja subfulva venom showed the presence of phospholipase A_2 enzymes by producing hemolytic haloes in indirect hemolytic assays due to formation of phospholipid hydrolysis products such as lysophospholipids and free fatty acids which are lytic. Different doses of aqueous extracts of *A. spinosus, C. articulatus, C. spinarum, C. molle* and *R. usambarensis* (Table 4.8) inhibited PLA_2 in a dose dependent manner. This inhibition may be due to the extracts' phytochemicals such as flavonoids, phenols, terpenoids and quinonoid activity which have been documented to deactivate snake venom constituents (Marcussi *et al*., 2007). Plant secondary metabolites react and sequester phospholipases and pose hindrance in binding of the latter to their target site (s) hence reverse anticoagulation action of phospholipase A_2 (Kini, 2003).

Procoagulant activity was studied using human citrated plasma but no coagulation of the plasma

was observed. This was in agreement with studies done by Suntravat *et al.* (2010), which showed that *N. subfulva* venom had no significant activity on both procoagulant and anticoagulant activities. *Naja subfulva* toxic phospholipase A_2 probably affects coagulation due to its interaction with plasma phospholipids.

The index of acute toxicity is the LD_{50} (Shatoor, 2011). According to Lorke (1983), substances with more toxic than 1 mg/kg body weight are highly toxic, while LD_{50} values greater than 5000 mg/kg body weight are of no practical interest. In the present studies, there were no mortalities observed up to the maximum dose level of 5000 mg/kg body weight of the aqueous extracts of *A. spinosus, C. articulatus, C. spinarum, C. molle* and *R. usambarensis* administered. Thus, the study suggests that aqueous extracts of *A. spinosus, C. articulatus, C. spinarum, C. molle* and *R. usambarensis* do not cause any apparent acute toxicity as the experimental animals tolerated the extracts without any symptoms of acute toxicity, even at larger doses and were therefore, considered safe for pharmacological treatment as indicated by Hodge and Sterner (2005) as well as Lorke (1983).

Subacute toxicity studies were conducted with repeated administration of 10, 300 and 1000 mg/kg body weight of aqueous extracts of *A. spinosus, C. articulatus, C. spinarum, C. molle* and *R. usambarensis* to investigate their effect on body weight, average body weight change and average weekly body weight change, organ weight, percent organ to body weight as well as hematological and biochemical parameters. Body weight alterations are indicators of serious effects of drugs and chemicals and quite significant if the body weight loss occurs and exceeds 10% of the initial body weight (Dybing *et al*., 2008). It is recommended that body weight be

measured at least once a week during toxicity studies because it is one of the most sensitive indicators of the condition of an animal if monitored frequently and carefully during a study (Summers *et al*., 2012). In the present study, the control mice gained a significantly greater weight compared to the extract-treated mice whose weight gain decreased as the doses increased from 10, 300 and 1000 mg/kg body weight sequentially. There was also a significant decrease ($\rho < 0.05$) in the average weekly body weight change in the extract-treated mice in a dose dependent manner for all the five studied aqueous plants extracts. The decreasing increase in body weights caused by the five studied aqueous plants extracts with the increase in extract dose could have been caused by the presence of flavonoids; alkaloids, tannins, steroids, and terpenoids present in these extracts that have previously been reported to cause suppression of appetite (Tucci 2010) through various mechanisms. Tucci (2010) reported that plants such as *Coleus forskohlii* contain terpenoids such as the diterpenoid, forskolin (7β-acetoxy-8, 13-epoxy-1α, 6β, 9α-trihydroxylabd-14-en-11-one), that has been shown to suppress appetite by activating adenylate cyclase which catalyzes the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP) (and pyrophosphate) which in turn promotes lipolysis, increases the basal metabolic rate, and increases the use of body fats. Other plants like *Caralluma fimbriata* and *Hoodia gordonii* contain steroids like pregnane glycosides such as 3β-[β-d-thevetopyranosyl-(1→4)-β-d-cymaropyranosyl-(1→4)-β-d-cymaropyranosyl-12-β-tigloyloxy-14β-hydroxy-pregn-5-en-20-one, which has been shown to suppress appetite using peripheral and central effects. In the adipose tissue, pregnane glycosides decrease lipogenesis. They act by amplifying the signalling of the energy sensing function in the hypothalamus and hence reduce intake of calories resulting in decreasing body weight. *Catha edulis* contain cathinone (1-α-aminopropiophenone) and cathine (D-*nor*-pseudoephedrine) which are

monoamine alkaloids which have been shown to suppress appetite by centrally acting on the hypothalamus. In addition to the central effects, cathinone enhances sympathomimetic activity leading to prolonging gastric emptying leading to a decrease in hunger and increasing fullness. Tea and coffee contain caffeine, a water soluble alkaloid, which is associated with appetite suppression via central and peripheral mechanisms due to its effects on adenosine A1, A2A and A2B receptors. This reduces the intake of calories and therefore energy intake, and exerting thermogenesis and lipolysis and when taken over a long time results in weight loss (Tucci 2010). Tea also contains flavonoids such as Catechins (flavan-3-ols) including (-)-epigallocatechin-3-gallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechin-3-gallate (ECG), and (-)-epicatechin (EC) which are associated with appetite suppression by reduction of calorie intake and hence reduction of energy intake by inhibiting small-intestine micelle formation and inhibition of α-glucosidases activity which leads to a decrease in digestion and absorption of carbohydrates such as glucose. Catechins are also associated with an increase in sympathetic nervous system activity, thermogenesis and fat oxidation which also leads to loss of weight (Tucci 2010).

The reduction of blood glucose in the extract-treated mice compared to that of the normal control mice with the increase in the extract doses from 10, 300 and 1000 mg/kg body weight could have been caused by first the presence of mineral elements (V, Cr, Zn, Se, Mg, Ca, K and Mn) and phytochemicals in the extract that promote the secretion and uptake of insulin in the blood stream which would promote glucose uptake into the cells and oxidation. Examples of such compounds include Glipizide, a sulfonylurea class of drugs that stimulates insulin secretion from β-cells and is used as an oral hypoglycemic agent in diabetic patients, and Metformin, a

biguanide drug, that corrects hyperglycemia by stimulating peripheral glucose disposal and blunting hepatic glucose production.

Secondly, the presence of compounds in the extracts that suppress appetite, prolong hunger and increase a filling of fullness resulting in reduced nutrients (carbohydrates, proteins, lipids, minerals and vitamins) uptake for digestion and absorption into the bloodstream including blood glucose. Examples of such compounds include tannins which are astringent and bitter in taste making food unpalatable and suppress appetite. Tannins are of two types including hydrolysable tannins and non-hydrolysable tannins or condensed tannins or proanthocyanidins. Tannins have the ability to stabilize proteins.

Thirdly, the presence of compounds in the extracts that inhibit the activity of α-glucosidases (α-glucosidases inhibitors) resulting in reduced digestion of starch a complex carbohydrate into simple sugars such as glucose and their absorption resulting in reduced blood glucose levels. The reduced blood glucose level does not seem to be caused by hypoxia resulting from anemia caused by reduced levels of RBC ($x10^{12}$/L) and HB (g/dL) because the levels of RBC ($x10^{12}$/L), and HB (g/dL) in mice treated with aqueous extracts of *A. spinosus*, *C. articulatus*, *C. molle* and *R. usambarensis* especially at 1000 mg/kg body weight were comparable to those of the normal control mice. Anemia induces hypoxia which promotes anaerobic glycolysis which is 100-fold faster than aerobic glycolysis which occurs via oxidative phosphorylation.

The hypoglycemic action of α-glucosidases inhibitors results from competitive, reversible, membrane-bound intestinal α-glucoside hydrolase enzymes. Liu *et al*. (2016) demonstrated *in*

vitro inhibition of α-glucosidase by aqueous extracts of Qingzhuan dark tea. Examples of previously reported phytochemical compounds with *in vitro* α-glucosidase inhibitory activity include Scrophuside isolated from the roots of *Scrophularia ningpoensis* Hemsl., (Hua *et al*., 2014), Ningposide I and Ningposide II (Iridoid glycosides) isolated from the roots of *Scrophularia ningpoensis* Hemsl., (Hua *et al*., 2014), Alisol F and Alisol B isolated from *Alismatis rhizom*a (Li and Qu, 2012), Chalcomoracin, Moracin C, Moracin D and Moracin N isolated from *Morus alba* (Yang *et al*., 2012) and Eleutherinoside A isolated from *Eleutherine americana* (Ieyama *et al*., 2011). Pancreatic α-amylase hydrolyzes complex starches to oligosaccharides in the lumen of the small intestine, while the membrane-bound intestinal α-glucosidases hydrolyze oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharides in the brush border of the small intestine. Examples of α-glucosidases inhibitors include acarbose, miglitol and voglibose which inhibit α-glucosidases in the brush border of the enterocytes lining of intestine and pancreatic α-amylases in the lumen of the intestines competitively. Pancreatic α-amylases digest complex starches to oligosaccharides, whereas sucrases, maltases, isomaltases hydrolyze oligosaccharides, trisacchrides and disaccharides into simple sugars including glucose.

Trojan-Rodrigues *et al*. (2011) isolated Kaempferol-3-neohesperidoside and demonstrated that it lowers blood glucose levels by mimicking the action of insulin. Kumar *et al*. (2012) isolated Bergenin from *Caesalpinia digyna* Rottler (Leguminosae) and demonstrated that it lowers blood glucose levels by regenerating pancreatic β cells in type 2 diabetic rats. Qiao *et al*. (2011) isolated Trans-tiliroside from *Potentilla chinesis* and demonstrated that it lowers blood glucose level in alloxan-induced diabetic mice and streptozotocin-induced diabetic rats. Nazreen *et al*.

(2012) isolated 5,7-dihydroxy-6,8-dimethyl-4′-methoxy flavone and 8-(2-hydroxypropan-2-yl)-5-hydroxy-7-methoxy-6-methyl4′-methoxy flavones from *Callistemon lanceolatus* DC (Myrtaceae) and demonstrated that they lower blood glucose level in streptozotocin induced diabetic rats. Further, 6-O-galloyl-5'-hydroxy mangiferin, mangiferin, 5-hydroxy mangiferin, and methyl gallate isolated from *Mangifera indica* were shown to lower blood glucose level in alloxan-induced diabetic rats (Md *et al*., 2013). It has previously been reported that phytochemicals such as phenols, flavonoids, alkaloids, tannins, and saponins which are present in these plants extracts cause blood glucose lowering effect (Elliot *et al*., 2000).

Reduced blood glucose implies decreased production of ATP through glycolysis, tricarboxylic acid cycle, electron transport chain and finally oxidative phosphorylation. As the rate of ATP production falls below the level required by membrane ion pumps for maintaining proper intracellular ion concentrations, osmotic balance is disrupted so that the cell and its membrane-enveloped organelles begin to swell. The resulting overstretched membranes become permeable, thereby leaking their enclosed contents (Voet, Voet and Pratt, 2017). This may account for the increased levels of ALT and GGT after treatment of mice with aqueous extracts of *A. spinosus*, *C. articulatus*, *C. molle* and *R. usambarensis* and an increase in percent organ to body weight of brain (after treatment of mice with extracts of *A. spinosus*, *C. molle* and *R. usambarensis*), liver (after treatment of mice with extracts of *A. spinosus*, *C. articulatus*, *C. molle* and *R. usambarensis*), lungs (after treatment of mice with *C. molle* extract) and spleen (after treatment of mice with *A. spinosus* extract).

Reduced blood glucose levels in the cells may also induce the body cells to result to other alternative sources of energy like tissue proteins and lipids both of which are components of cell membranes of tissues and organs. Catabolism of membrane proteins after cleavage from cell membranes results in the release of amino acids which on catabolism via deamination may result in intermediates of glycolysis and tricarboxylic acid cycle that can be used for glucose biosynthesis, provision of energy or intermediates that can be used for biosynthesis of ketone bodies while catabolism of lipids after cleavage from cell membranes results in fatty acids that are catabolized via β-oxidation to ketone bodies acetoacetate, β-hydroxybutyrate and acetone. Acetoacetate and β-hydroxybutyrate are weak acids which may also contribute to acidity due to ketoacidosis. This plucking of proteins and lipids from cell membranes of the tissues and organs interferes with membrane integrity and therefore also contributes to swelling of organs due to intake of extracellular fluids and leakage of intracellular components out of the cells including proteins such as ALT and GGT leading to their increase in serum-plasma. The degradation of tissue and organ cell membrane proteins and lipids may also lead to wasting and decrease in body weight (hence muscle mass) and creatinine (CREAT). CREAT which is proportional to the muscle mass is a degradation product of phosphocreatine in muscle. This explanation seems viable considering that the percent organ to body weight of the kidney was unaffected by treatment of mice with the plants extracts (Voet, Voet and Pratt, 2017).

However, the non-significant effect on the blood glucose levels caused by the treatment of mice with aqueous extracts of *C. spinarum* at 10 and 300 mg/kg body weight may have resulted from the reduction of the levels of RBC ($x10^{12}$/L), HB (g/dL) and PCV (%) which may induce anemia in mice resulting in reduced oxygen in the cells (hypoxia) and hence promoting anaerobic

catabolism of glucose leading to the production of lactic acid creating an acidic environment in the cells. Anaerobic glycolysis consumes glucose and generates ATP one hundred times faster than aerobic glycolysis which generates ATP through oxidative phosphorylation and hence results in blood glucose reduction; blood glucose reduction was not observed in this study with *C. spinarum* extract-treated mice in this study. Under anaerobic conditions in muscle, pyruvate is reduced to lactate to regenerate NAD^+ in a process known as homolactic fermentation. The decreased intracellular pH that accompanies anaerobic glycolysis (because of lactic acid production) permits the released lysosomal enzymes (which are active only at acidic pH) to degrade the cell contents. Thus, O_2 deprivation leads not only to cessation of cellular activity but to irreversible cell damage and cell death. Rapidly respiring tissues, such as the heart and brain, are particularly susceptible to damage by oxygen deprivation (Voet and Voet, 2016). This hypoxic state for mice treated with aqueous extract of *C. spinarum* may also account for the increase in the percent organ to body weight of the liver, brain, heart, and spleen due to fluids intake especially significant at 1000 mg/kg body weight compared to that of the normal control mice.

The increase in the percent organ to body weight of the brain, liver, lungs, spleen and heart in *C. spinarum* extract-treated mice may indicate increased intake of extracellular fluids due to reduced membrane integrity of the cells caused by the overdose of lead. Lead overdose in the blood inhibits the enzymes of heme synthesis [δ-aminolevulinate dehydratase], thiol-containing antioxidants such as oxidized (GSSG) and reduced (GSH) glutathione, and enzymes such as superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, and glutathione-s-transferase leading to increased levels of free radicals (ROS) like hydroperoxides ($H_2O^{\cdot}$),

hydrogen peroxide (H_2O_2), singlet oxygen, superoxide radical, and hydroxyl radical and depletion of antioxidant reserves which destroy the generated reactive oxygen species (ROS) which interfere with membrane integrity of tissues, organs and organelles and damage the cells. These antioxidant reserves include reduced glutathione (GSH) which quenches free radicals and oxidized glutathione (GSSG). The formation of oxidized glutathione (GSSG) from reduced glutathione (GSH) catalyzed by glutathione peroxidase releases reducing equivalents ($H^+ + e$) from its thiol groups present in cysteine residues to ROS and stabilizes them. Oxidized glutathione (GSSG) is converted to reduced glutathione (GSH) by glutathione reductase. Under conditions of oxidative stress, the concentration of GSSG is higher than that of GSH (Flora *et al*., 2012).

Further, lead forms covalent attachments with sulfhydryl groups present in antioxidant enzymes such as glutathione reductase, glutathione peroxidase, and glutathione-s-transferase which are its targets and inactivates them. This results in the synthesis of glutathione from cysteine via the γ-glutamyl cycle which is inadequate to replenish the supply for GSH. Lead also inactivates superoxide dismutase, and catalase leading to reduced ability to dispose superoxide radicals and scavenging of hydrogen peroxide. Lead also replaces bivalent cations such as Zn^{2+}, Ca^{2+}, Mg^{2+}, Fe^{2+} and monovalent cations such as Na^+ which are cofactors of antioxidant enzymes inactivating them (Flora *et al*., 2012).

Lead overdose in blood also causes hemoglobin oxidation which directly causes red blood cell hemolysis as observed in this study with *C. spinarum* extract by inhibiting δ-aminolevulinate dehydratase the enzyme involved in hemoglobin synthesis causing accumulation of its substrates

in both blood and urine. The accumulated substrates lead to the generation of free radical hydrogen peroxide and superoxide radical and also interact with oxyhemoglobin resulting in the production of hydroxyl radical. The generated free radicals capture electrons from lipids present on the cell membranes and the membranes of the organelles inside the cell membrane interfering with their integrity and damaging them. This increase in the percent organ to body weight of the liver and spleen due to the entry of extracellular fluids may be responsible for the decrease in T-BIL. Bilirubin is a catabolic product of hemoglobin which occurs in the reticuloendothelial cells (which are made of cells descending from the monocytes with the ability to phagocytose foreign materials and particles) that line the sinusoids of the liver, spleen and the bone marrow, and the reticular cells of the lymphatic tissue (macrophages) and bone marrow (fibroblasts). The plants extracts may be decreasing the functioning of the reticuloendothelial cells either by causing abnormalities in them or interfering with their mechanism of control resulting in decreased degradation of hemoglobin to bilirubin. The increase in monocytes levels may not explain the reduced degradation of hemoglobin to bilirubin (Flora *et al*., 2012).

The increase in the percent organ to body weight of the liver (liver damage) may also have been caused by the decreased levels of BUN in the extract-treated mice in addition to their reduced appetite resulting in decreased body weight gain. Urea is synthesized in the liver (Voet, Voet and Pratt, 2017). Lead toxicity caused by the provision of more lead than iron as observed in this study with aqueous extracts of *C. spinarum* at 10 and 300 mg/kg body weight inhibits the production erythropoietin, a hormone produced by the kidneys whose function is to stimulate the production of red blood cells by the bone marrow (Flora *et al*., 2012). Overdose of V (*R. usambarensis, A. spinosus, C. molle, C. articulatus*), Mn (*R. usambarensis, C. molle, C.*

articulatus), Cr (*A. spinosus*, *C. articulatus*), Fe (*A. spinosus*, *C. articulatus*), and Pb (*C. spinarum, C. molle*) may also be responsible of the toxicity evidenced by alterations in the levels of analytes and enzymes, average weekly body weight changes, and percent organ to body weight changes observed in the extract-treated mice in this study. Overdose of these mineral elements cause oxidative stress by inducing an imbalance between the production of reactive oxygen such as superoxide ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$) and reactive nitrogen species such as nitric oxide ($^{\bullet}NO$), peroxynitrite ($ONOO^-$) and nitrogen dioxide ($^{\bullet}NO_2$), and defense antioxidants (reduced glutathione (GSH) and oxidized glutathione (GSSG)) whose production is decreased. The excess mineral elements inhibit the activities of antioxidant enzymes such as glutathione reductase, glutathione peroxidase, and glutathione-s-transferase which are responsible for the metabolism of glutathione, and superoxide dismutase (SOD) and catalase which are responsible for the removal of superoxide ($^{\bullet}O_2^-$) radical and hydrogen peroxide (H_2O_2), respectively. This results in high levels of reactive oxygen and nitrogen species and low levels of antioxidants, a situation which damages and kills cells (Jaishankar *et al*., 2014).

Chromium in blood exists as Cr (II), Cr (III), Cr (IV), Cr (V) and Cr (VI) with Cr (III) and Cr (VI) being the most stable form. Cr (III) is harmless because it is less permeable than Cr (VI) which penetrates cell membranes through passages of isoelectrical and isostructural anions such as SO_4^{2-} and HPO_4^{2-} channels. These chromates are taken up through phagocytosis. Cr (VI) is a strong oxidizing agent which is reduced to produce Cr (V) and Cr (IV) which are different from Cr (III). Stabilization of Cr (V) is carried out by glutathione. Reduction of intracellular Cr (VI) is a detoxification mechanism when this reduction occurs away from the target site. However, if this intracellular Cr (VI) reduction occurs near the target site, it activates Cr. The reaction

between Cr (VI) and biological reductants such as thiols and ascorbate result in the production of reactive oxygen and nitrogen species leading to oxidative stress in cells which cause damage to DNA through the formation of adducts, chromosomal aberrations, sister chromatid exchanges, alterations in replication and transcription of DNA, and proteins. Excess chromium in the blood inhibits erythrocyte glutathione reductase which reduces the capacity to convert methemoglobin to hemoglobin (Jaishankar *et al*., 2014).

Iron is a component of enzymes such as cytochromes and catalase as well as oxygen transport proteins like hemoglobin and myoglobin. It is interconvertible between Fe^{2+} and Fe^{3+}. Excess iron beyond what binds to the iron-transport protein affects the concentration of iron in cells and fluids. This excess unbound iron is corrosive to the gastrointestinal tract and biological fluids and enters into cells of heart, liver and brain, disrupting oxidative phosphorylation (Li *et al*., 2017). The conversion of Fe^{2+} to Fe^{3+} releases H^+ ions and electrons and thus increasing metabolic acidity. The H^+ ions and electrons participate in lipid peroxidation which results with severe damage to mitochondrion, microsomes, and other cellular organelles. Excess free iron promotes the production of reactive oxygen and nitrogen species such as superoxide, and hydrogen peroxide, which are neutralized by superoxide dismutase, catalase and glutathione peroxidase. The superoxide radical can release free iron from ferritin which reacts with more superoxide and hydrogen peroxide to produce the hydroxyl radical which is more toxic. Hydroxyl radicals inactivate certain enzymes, initiate lipid peroxidation, depolymerize polysaccharides and cause DNA damage resulting in cellular mutations and malignant transformations which in turn cause an array of diseases including cell death. Excess mineral elements in blood such as chromium, iron, lead, manganese, and vanadium can lower energy levels and damage the functioning of the

brain, lungs, kidney, liver, blood composition and other important organs (Juranovic *et al*., 2015). Long-term exposure can lead to physical, muscular, and neurological degenerative processes that imitate diseases such as multiple sclerosis, Parkinson's disease, Alzheimer's disease and muscular dystrophy. Repeated long-term exposure of some metals and their compounds may even cause cancer (Jaishankar *et al*., 2014).

Cardiac glycosides present in the aqueous extracts of *C. spinarum* and *R. usambarensis* could have been responsible for the increase in the percent organ to body weight of the heart and the decrease in the average weekly body weight change in mice treated with these two plants extracts at 10, 300 and 1000 mg/kg body weight. Cardiac glycosides affects the K^+/Na^+-ATPase pump in cardiac muscle cells to alter their function of pumping K^+ ions in and Na^+ ions out. They inhibit the K^+/Na^+-ATPase pump so that Na^+ ions are not extruded resulting in increasing the intracellular Na^+ ions. Increased intracellular Na^+ ions inhibit the function of Na^+/Ca^{2+}-exchanger which pumps Ca^{2+} ions out of the cell and Na^+ ions in at a ratio of $3Na^+/Ca^{2+}$. Calcium ions are also not extruded and build up inside the cells as well. The disrupted Ca^{2+} ions homeostasis and increased cytoplasmic Ca^{2+} ions concentration cause increased Ca^{2+} ions uptake into the sarcoplasmic reticulum. Increased Ca^{2+} ions store in the sarcoplasmic reticulum allow greater Ca^{2+} ions release on stimulation so that myocytes achieve faster and more powerful contractions by cross-bridge cycling. The refractory period of the atrioventricular node (part of the electrical conduction system of the heart that coordinates the top of the heart. It electrically connects the atria and ventricles) is increased so cardiac glycosides also decrease the heart rate. For example, excess cardiac glycosides result in cardiac contractions with greater force as more calcium is released from sarcoplasmic reticulum of cardiac muscle cells (Haydock, 2012).

Toxicity also results in changes to heart ionotropic (a change in the force of contraction (such as in heart muscle) produced by a transmitter substance or a hormone) activity resulting in multiple kinds of dysrhythmias (faster [tachycardia] or slower [bradycardia]) and potentially fatal ventricular tachycardia (a type of regular fast heart rate that arises from improper electrical activity in the ventricles of the heart (which are dangerous when they occur for prolonged periods). These dysrhythmias are an effect of influx of sodium and decrease in resting membrane potential threshold in cardiac muscle cells. When taken beyond a narrow dosage range specific for each cardiac glycoside, they rapidly become dangerous by interfering with fundamental processes that regulate membrane potential (the difference in electrical potential between the interior and the exterior of a biological cell). They are toxic to the heart, brain and the gut at doses that are easily reached. In the heart, the most negative effect is premature ventricular contraction (Haydock, 2012).

The increased white blood cell (WBC $x10^{12}$/L) and differential white blood cell count including NEU ($x10^{9}$/L), LYM ($x10^{9}$/L), and MON ($x10^{9}$/L) induced by the treatment of mice with aqueous extracts of *A. spinosus, C. articulatus*, *C. molle* and *R. usambarensis* in the subacute toxicity study indicates increased immunity contributed by some phytoconstituents including vitamins C, D, A, E, B6, B12, and folate and mineral elements zinc, iron, copper and selenium present in these extracts. However, the levels of these specific vitamins in these plants extracts were not estimated in this study though they are commonly present in plants. This implies that these plants extracts can be used as drugs for immune boosting (immunomodulatory drugs).

Vitamin C in innate immunity is an effective antioxidant that protects against reactive oxygen species (ROS) and reactive nitrogen species (RNS) produced when pathogens are killed by immune cells; regenerates other important antioxidants such as glutathione and vitamin E to their active state; promotes collagen synthesis, thereby supporting the integrity of epithelial barriers; stimulates production, function and movement of leukocytes (such as neutrophils, lymphocytes, phagocytes); increases serum levels of complement proteins; has roles in antimicrobial and NK cell activities and chemotaxis; involved in apoptosis and clearance of spent neutrophils from sites of infection by macrophages), and in adaptive immunity can increase serum levels of antibodies; and has roles in lymphocyte differentiation and proliferation (Maggini *et al*., 2018).

Vitamin D in innate immunity causes vitamin D receptor to be expressed in innate immune cells (such as monocytes, macrophages, dendritic cells); increases the differentiation of monocytes to macrophages; stimulates immune cell proliferation and cytokine production and helps protect against infection caused by pathogens; 1,25-dihydroxyvitamin D3, the active form of vitamin D, regulates the antimicrobial proteins cathelicidin and defensin, which can directly kill pathogens, especially bacteria), and in adaptive immunity has mainly inhibitory effect; for example, 1, 25-dihydroxyvitamin D3 suppresses antibody production by B cells and inhibits T cell proliferation (Maggini *et al*., 2018).

Vitamin A in innate immunity helps in maintaining the structural and functional integrity of mucosal cells in innate barriers (such as skin, respiratory tract, etc.); important for normal functioning of innate immune cells (such as NK cells, macrophages, neutrophils), and in adaptive immunity is necessary for proper functioning of T and B lymphocytes, and thus for generation of

antibody responses to antigen; and involved in development and differentiation of Th1 and Th2 cells and supports Th2 anti-inflammatory response (Maggini *et al*., 2018).

Vitamin E in innate immunity is an important fat-soluble antioxidant; protects the integrity of cell membranes from damage caused by free radicals; and enhances IL-2 production and NK cell cytotoxic activity, and in adaptive immunity enhances T cell-mediated functions and lymphocyte proliferation; and optimizes and enhances Th1 and suppresses Th2 response (Maggini *et al*., 2018), vitamin B6 in innate immunity helps in regulating inflammation; has roles in cytokine production and NK cell activity and in adaptive immunity is required in the endogenous synthesis and metabolism of amino acids, the building blocks of cytokines and antibodies; has roles in lymphocyte proliferation, differentiation and maturation; maintains Th1 immune response; and has roles in antibody production (Maggini *et al*., 2018).

Vitamin B12 in innate immunity has roles in NK cell functions; folate which maintains innate immunity (NK cells) and in adaptive immunity may act as an immunomodulator for cellular immunity, especially with effects on cytotoxic cells (NK cells, CD8+ T-cells); facilitates production of T lymphocytes; and involved in humoral and cellular immunity and one-carbon metabolism (interactions with folate) (Maggini *et al*., 2018), and folate in innate immunity maintains innate immunity (NK cells) and in adaptive immunity has roles in cell-mediated immunity; important for sufficient antibody response to antigens; and supports Th1-mediated immune response (Maggini *et al*., 2018).

Mineral elements including iron in innate immunity is involved in regulation of cytokine production and action; forms highly-toxic hydroxyl radicals, thus is involved in the process of killing bacteria by neutrophils; and is important in the generation of reactive oxygen species (ROS) that kill pathogens and in adaptive immunity is important in the differentiation and proliferation of T lymphocytes; and is essential for cell differentiation and growth, component of enzymes critical for functioning of immune cells (such as ribonucleotide reductase involved in DNA synthesis) (Maggini *et al*., 2018); selenium in innate immunity is essential for the function of selenium-dependent enzymes (selenoproteins) that can act as redox regulators and cellular antioxidants, potentially counteracting ROS. Selenoproteins are important for the antioxidant host defense system affecting leukocyte and NK cell function, and in adaptive immunity is involved in T lymphocyte proliferation; and has roles in the humoral system (such as immunoglobulin production) (Maggini *et al*., 2018), zinc in innate immunity has antioxidant effects by protecting against ROS and reactive nitrogen species (RNS); helps in modulating cytokine release and induces proliferation of $CD8^+$ T cells; helps in maintaining skin and mucosal membrane integrity and in adaptive immunity has a central role in cellular growth and differentiation of immune cells that have a rapid differentiation and turnover; essential for intracellular binding of tyrosine kinase to T cell receptors, required for T lymphocyte development and activation; and supports Th1 response (Maggini *et al*., 2018), and copper in innate immunity is a free-radical scavenger; has antimicrobial properties; accumulates at sites of inflammation important for IL-2 production and response; and may play a role in the innate immune response to bacterial infections, and in adaptive immunity has roles in T cell proliferation; antibody production and cellular immunity (Maggini *et al*., 2018).

5.2 CONCLUSIONS

i) All the five aqueous plants extracts studied demonstrated significant antivenom activity both in *in vivo* and *in vitro* assays.

ii) Acute toxicity demonstrated no significant toxicity while subacute toxicity studies showed significant toxicity of the five aqueous plants extracts based on the average weekly body weight changes, percent organ to body weight, and biochemical parameters.

iii) Qualitative phytochemical analysis of the five aqueous plants extracts showed presence of tannins, cardiac glycosides, phenols, triterpenoids, terpenoids, saponins, alkaloids and flavonoids that could have contributed to the plants ability to neutralize snake venom and also contributed to the observed toxicity.

iv) Quantitative mineral element analysis of the extracts revealed presence of Ni, K, Hg, Cu, Rb, Se, Br, Br, Ti, Zn, As, and Ca at different concentrations below the recommended daily allowances and may therefore not be responsible of the observed toxicity but may be responsible for the observed antivenom activity. However, five of the estimated mineral element including Fe (*C. articulatus*, *A. spinosus*), V (*R. usambarensis*, *C. molle*, *C. articulatus*, *A. spinosus*), Cr (*C. articulatus, A. spinosus*), Mn (*R. usambarensis*, *C. molle*, *Cyperus articulatus*) and Pb (*C. molle*, *C. spinarum*) were present in the five aqueous plants extracts above the recommended daily allowance/intake and could be responsible for the observed toxicity.

5.3 RECOMMENDATION

The studied herbs from Turkana and Uasin-Gishu counties are recommended for continued use in treatment of snakebites at the tested doses where conventional antivenoms are not available.

5.3.1 RECOMMENDATIONS FOR FURTHER STUDIES

i) The plant extracts should be tested against venoms from different snake species and using more protocols that would include: hemorrhagic, fibrinolytic, and edema forming activities.

ii) Further, isolation, purification and structural elucidation of active constituents from the plant extracts need to be undertaken.

iii) Plant mineral elements need to be tested on their antisnake venom effects.

iv) Toxicity profile should be carried out using more biomarkers including those of organs whose integrity was not investigated after extracts administration.

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APPENDICES

APPENDIX 1: *Naja subfulva* probit analysis

Probit Analysis: Mice killed, Total mice versus Log dose
Distribution: Normal
Response Information

Variable	Value	Count
Mice killed	Event	16
	Non-event	9
Total mice	Total	25

Estimation Method: Maximum Likelihood
Regression Table

Variable	Coef	Standard Error	Z	P
Constant	0.0211204	0.322407	0.07	0.948
Log dose	4.09216	1.39612	2.93	0.003
Natural Response	0			

Log-Likelihood = -10.700
Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	2.33828	3	0.505
Deviance	2.93623	3	0.402

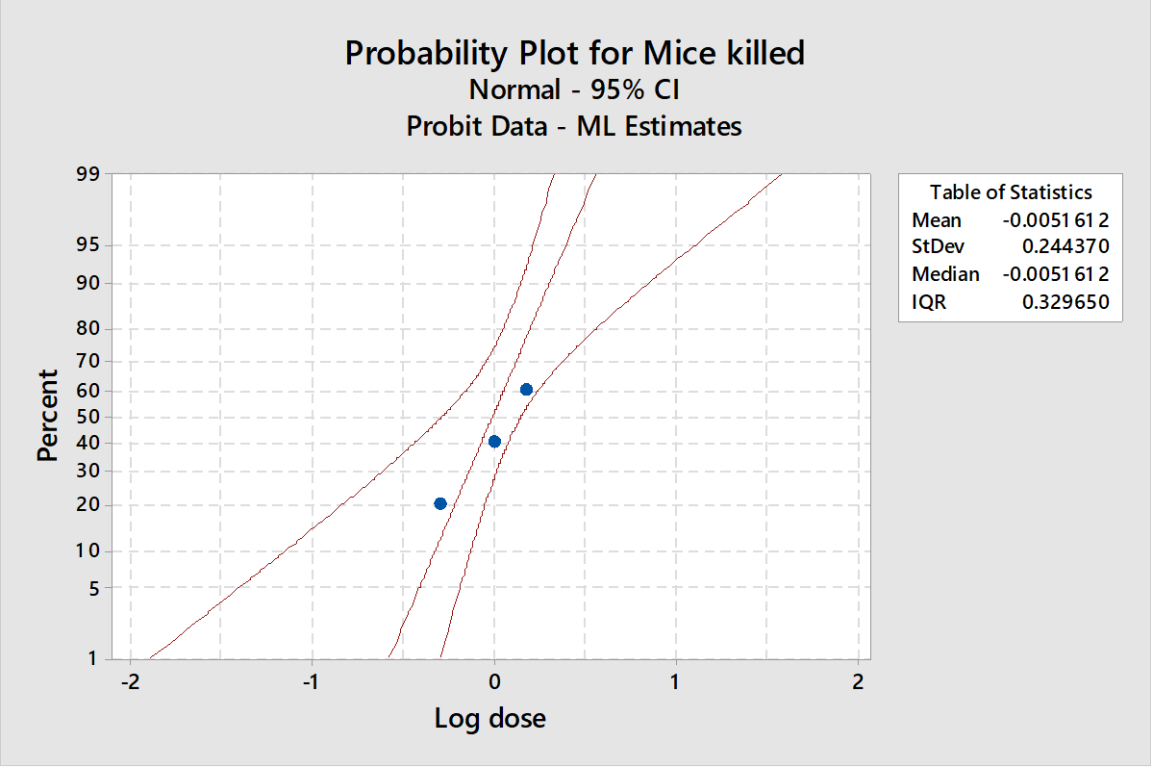
Tolerance Distribution
Parameter Estimates

Parameter	Estimate	Standard Error	95.0% Normal CI Lower	Upper
Mean	-0.0051612	0.0793235	-0.160632	0.150310
StDev	0.244370	0.0833717	0.125211	0.476927

Table of Percentiles

Percent	Percentile	Standard Error	95.0% Fiducial CI Lower	Upper
1	-0.573650	0.231524	-1.89380	-0.291523
2	-0.507035	0.210162	-1.69553	-0.248816
3	-0.464770	0.196767	-1.57007	-0.221380
4	-0.432976	0.186791	-1.47591	-0.200524
5	-0.407114	0.178752	-1.39948	-0.183394
6	-0.385101	0.171969	-1.33456	-0.168680
7	-0.365800	0.166074	-1.27776	-0.155662
8	-0.348518	0.160841	-1.22700	-0.143904
9	-0.332801	0.156122	-1.18093	-0.133117
10	-0.318334	0.151817	-1.13861	-0.123100
20	-0.210828	0.121337	-0.827789	-0.0450192

30	-0.133309	0.101926	-0.610380	0.0179990
40	-0.0670715	0.0882840	-0.433491	0.0807244
50	-0.0051612	0.0793235	-0.281726	0.152922
60	0.0567492	0.0753834	-0.151340	0.246498
70	0.122986	0.0775790	-0.0418735	0.376646
80	0.200506	0.0877989	0.0525878	0.562611
90	0.308011	0.111211	0.150502	0.853601
91	0.322479	0.114893	0.162093	0.894347
92	0.338196	0.118995	0.174408	0.938889
93	0.355477	0.123617	0.187655	0.988159
94	0.374778	0.128903	0.202133	1.04350
95	0.396791	0.135070	0.218296	1.10697
96	0.422653	0.142481	0.236881	1.18194
97	0.454448	0.151798	0.259233	1.27461
98	0.496713	0.164475	0.288268	1.39847
99	0.563328	0.184975	0.332856	1.59486



APPENDIX 2: *Amaranthus spinosus* probit analysis

Probit Analysis: Mice killed, Number of mice versus Log dose
Distribution: Normal
Response Information

Variable	Value	Count
Mice killed	Event	4
	Non-event	21
Number of mice	Total	25

Estimation Method: Maximum Likelihood
Regression Table

Variable	Coef	Standard Error	Z	P
Constant	4.85466	3.14658	1.54	0.123
Log dose	-2.49960	1.36336	-1.83	0.067
Natural Response	0			

Log-Likelihood = -9.082
Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	1.20782	3	0.751
Deviance	1.42676	3	0.699

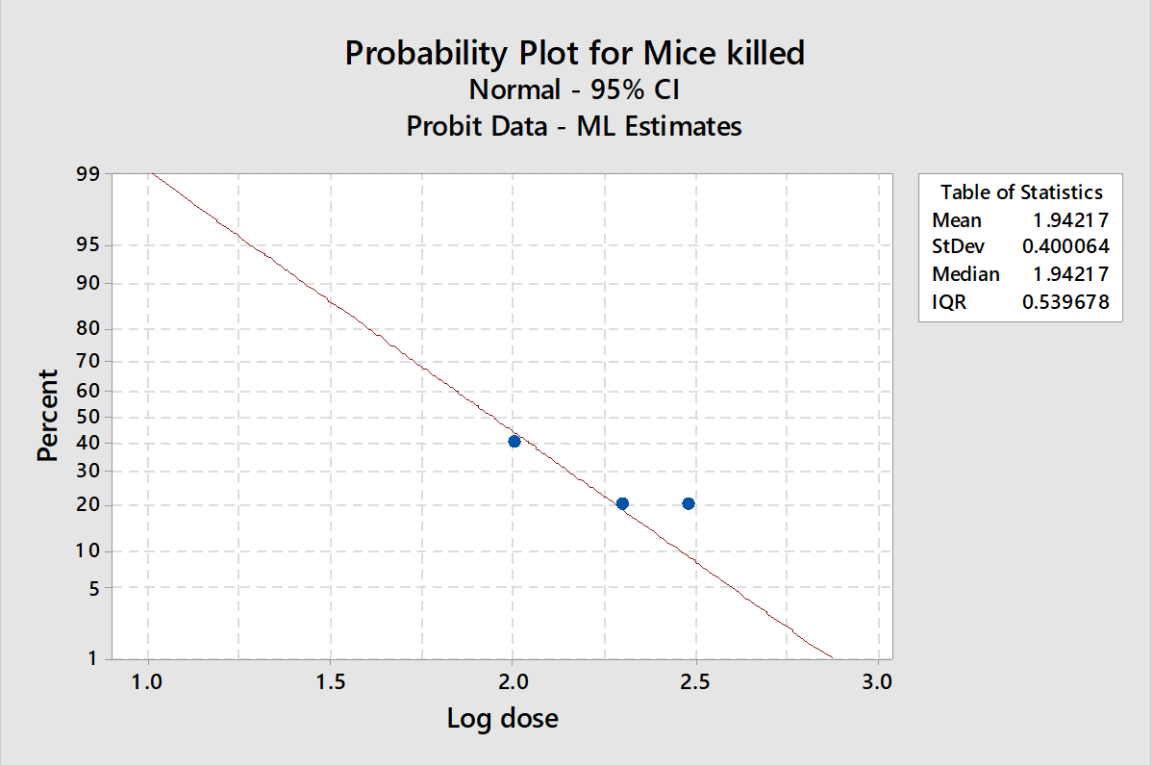
Tolerance Distribution
Parameter Estimates

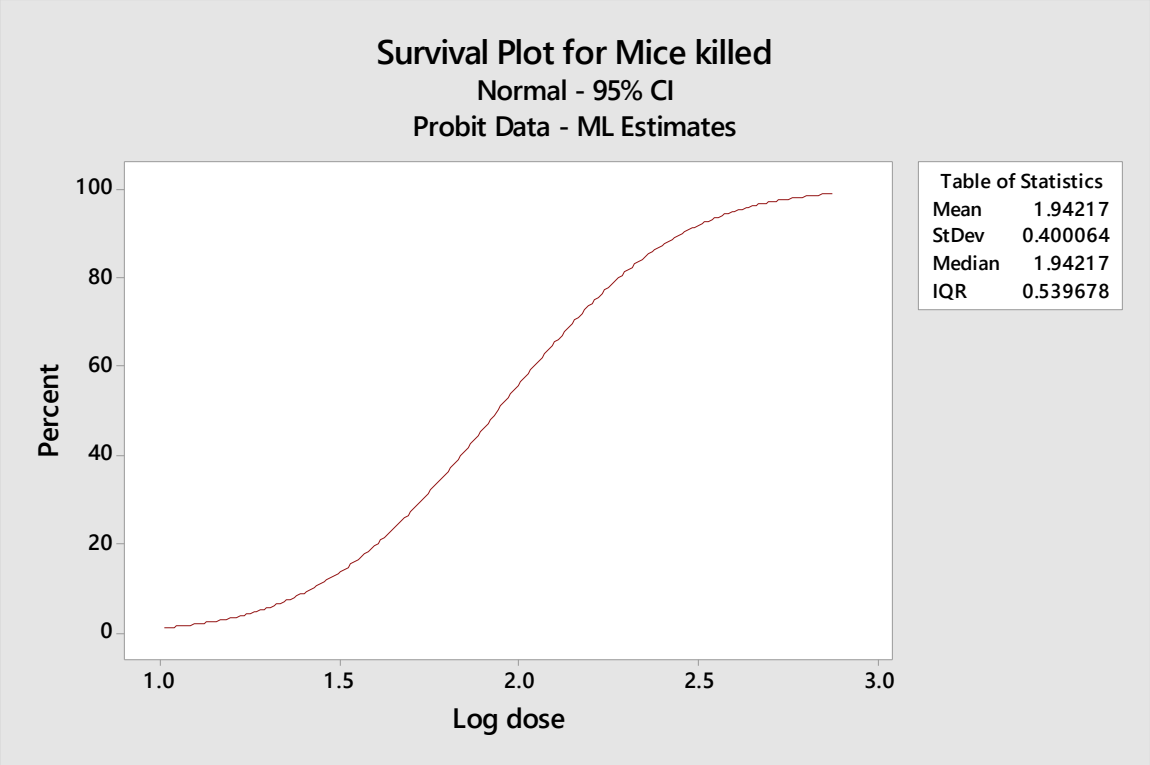
Parameter	Estimate	Standard Error	95.0% Normal CI Lower	Upper
Mean	1.94217	0.233834	1.48387	2.40048
StDev	0.400064	0.218208	0.137359	1.16520

Table of Percentiles

Percent	Percentile	Standard Error	95.0% Fiducial CI Lower	Upper
1	2.87286	0.341967	*	*
2	2.76380	0.288077	*	*
3	2.69461	0.255177	*	*
4	2.64256	0.231404	*	*
5	2.60022	0.212903	*	*
6	2.56418	0.197917	*	*
7	2.53258	0.185496	*	*
8	2.50429	0.175068	*	*
9	2.47856	0.166261	*	*
10	2.45488	0.158820	*	*
20	2.27888	0.133062	*	*

30	2.15197	0.154014	*	*
40	2.04353	0.190924	*	*
50	1.94217	0.233834	*	*
60	1.84082	0.281099	*	*
70	1.73238	0.334400	*	*
80	1.60547	0.398879	*	*
90	1.42947	0.490443	*	*
91	1.40579	0.502892	*	*
92	1.38006	0.516441	*	*
93	1.35176	0.531369	*	*
94	1.32017	0.548074	*	*
95	1.28413	0.567165	*	*
96	1.24179	0.589641	*	*
97	1.18974	0.617336	*	*
98	1.12054	0.654243	*	*
99	1.01149	0.712591	*	*




Survival Plot for Mice killed
Normal - 95% CI
Probit Data - ML Estimates
Percent
100
80
60
40
20
0
1.0
1.5
2.0
2.5
3.0
Log dose
Table of Statistics
Mean 1.94217
StDev 0.400064
Median 1.94217
IQR 0.539678

APPENDIX 3: *Carissa spinarum*

Probit Analysis: Mice killed, Number of mice versus Log dose
Distribution: Normal
Response Information

Variable	Value	Count
Mice killed	Event	5
	Non-event	20
Number of mice	Total	25

Estimation Method: Maximum Likelihood
Regression Table

Variable	Coef	Standard Error	Z	P
Constant	3.02423	2.79792	1.08	0.280
Log dose	-1.62768	1.18166	-1.38	0.168
Natural Response	0			

Log-Likelihood = -11.526
Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	0.94913	3	0.814
Deviance	1.31068	3	0.727

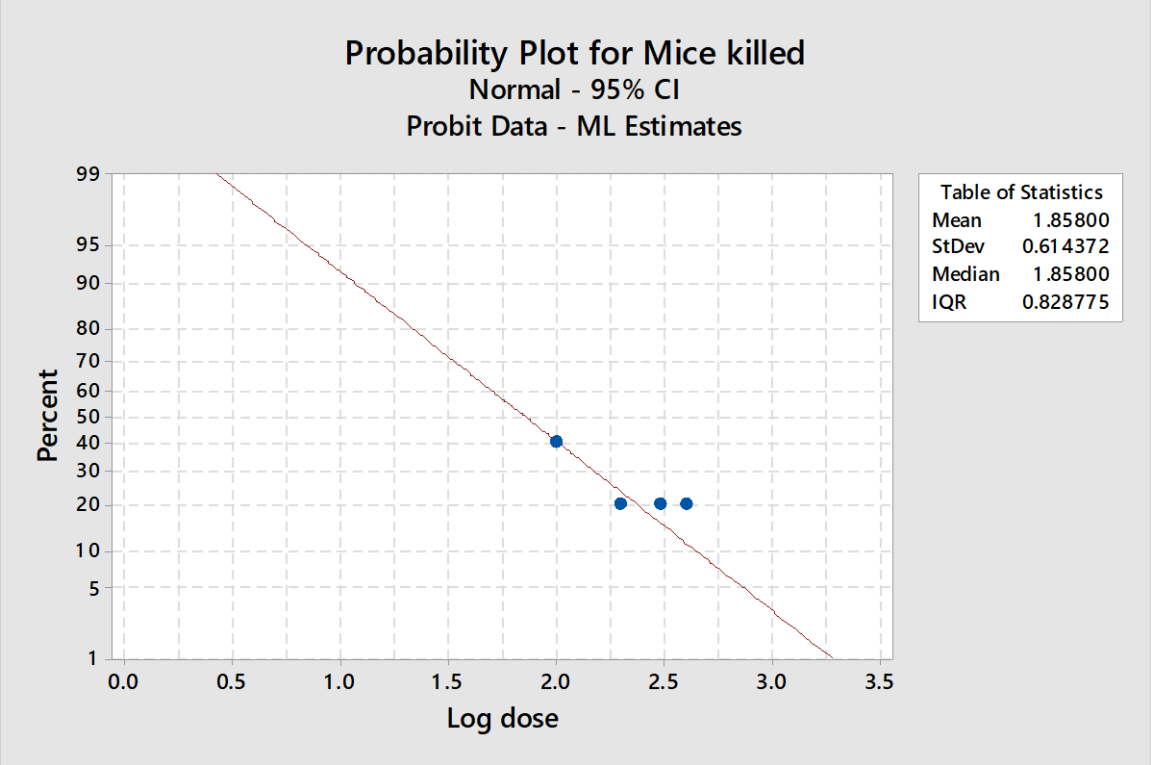
Tolerance Distribution
Parameter Estimates

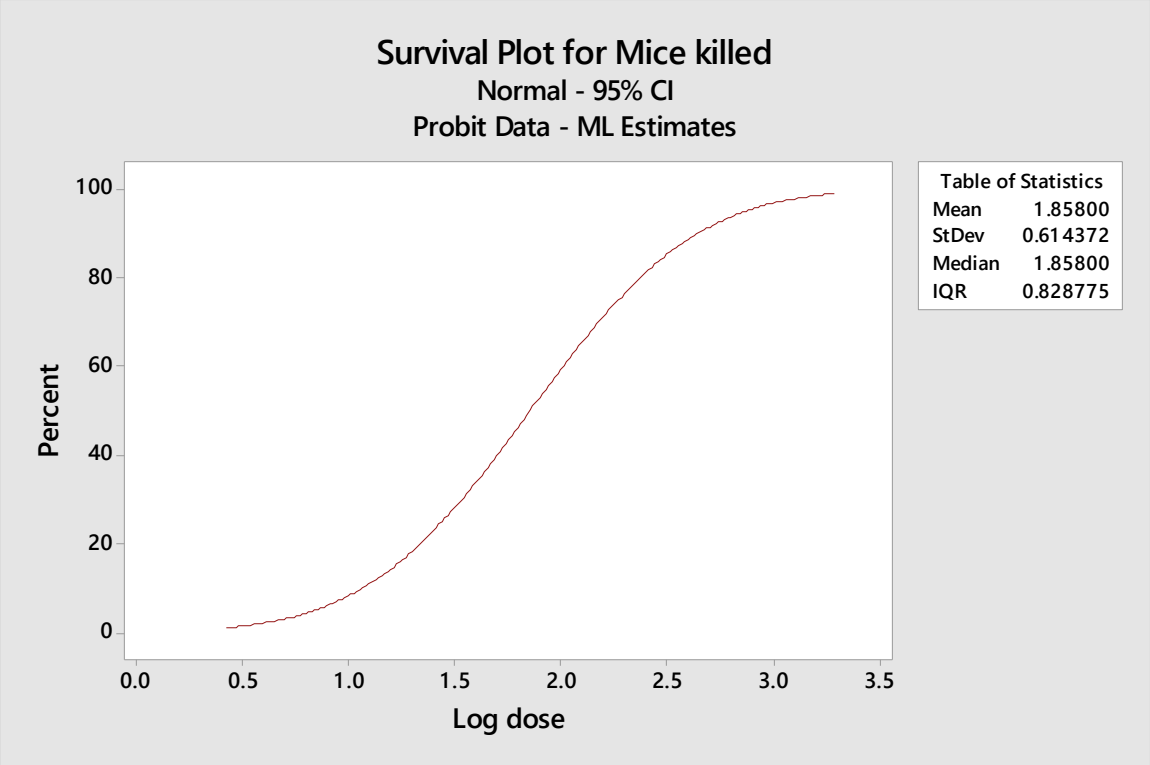
Parameter	Estimate	Standard Error	95.0% Normal CI Lower	Upper
Mean	1.85800	0.403949	1.06628	2.64973
StDev	0.614372	0.446020	0.148074	2.54908

Table of Percentiles

Percent	Percentile	Standard Error	95.0% Fiducial CI Lower	Upper
1	3.28725	0.701368	*	*
2	3.11977	0.584827	*	*
3	3.01351	0.512102	*	*
4	2.93358	0.458348	*	*
5	2.86856	0.415472	*	*
6	2.81321	0.379787	*	*
7	2.76469	0.349303	*	*
8	2.72124	0.322828	*	*
9	2.68173	0.299603	*	*
10	2.64535	0.279124	*	*
20	2.37507	0.183095	*	*

30	2.18018	0.222033	*	*
40	2.01365	0.307406	*	*
50	1.85800	0.403949	*	*
60	1.70235	0.507335	*	*
70	1.53583	0.621662	*	*
80	1.34094	0.758054	*	*
90	1.07065	0.949677	*	*
91	1.03428	0.975604	*	*
92	0.994767	1.00380	*	*
93	0.951319	1.03483	*	*
94	0.902795	1.06953	*	*
95	0.847452	1.10913	*	*
96	0.782432	1.15572	*	*
97	0.702497	1.21306	*	*
98	0.596238	1.28937	*	*
99	0.428761	1.40984	*	*




Survival Plot for Mice killed
Normal - 95% CI
Probit Data - ML Estimates
Percent
100
80
60
40
20
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
Log dose
Table of Statistics
Mean 1.85800
StDev 0.614372
Median 1.85800
IQR 0.828775

APPENDIX 4: *Combretum molle* probit analysis

Probit Analysis: Mice killed, Total mice versus Log dose
Distribution: Normal
Response Information

Variable	Value	Count
Mice killed	Event	6
	Non-event	19
Total mice	Total	25

Estimation Method: Maximum Likelihood
Regression Table

Variable	Coef	Standard Error	Z	P
Constant	5.01474	2.83458	1.77	0.077
Log dose	-2.41508	1.20129	-2.01	0.044
Natural Response	0			

Log-Likelihood = -11.563
Goodness-of-Fit Tests

Method	Chi-Square	DF	P
Pearson	1.13714	3	0.768
Deviance	1.38400	3	0.709

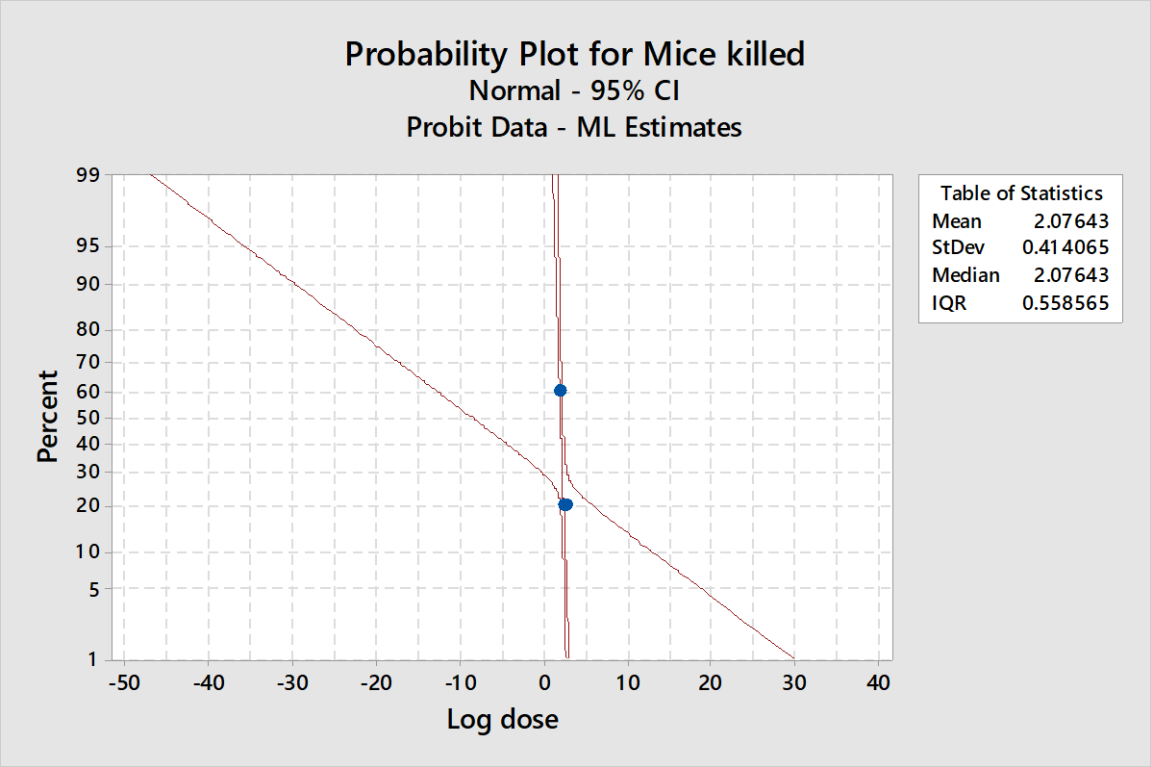
Tolerance Distribution
Parameter Estimates

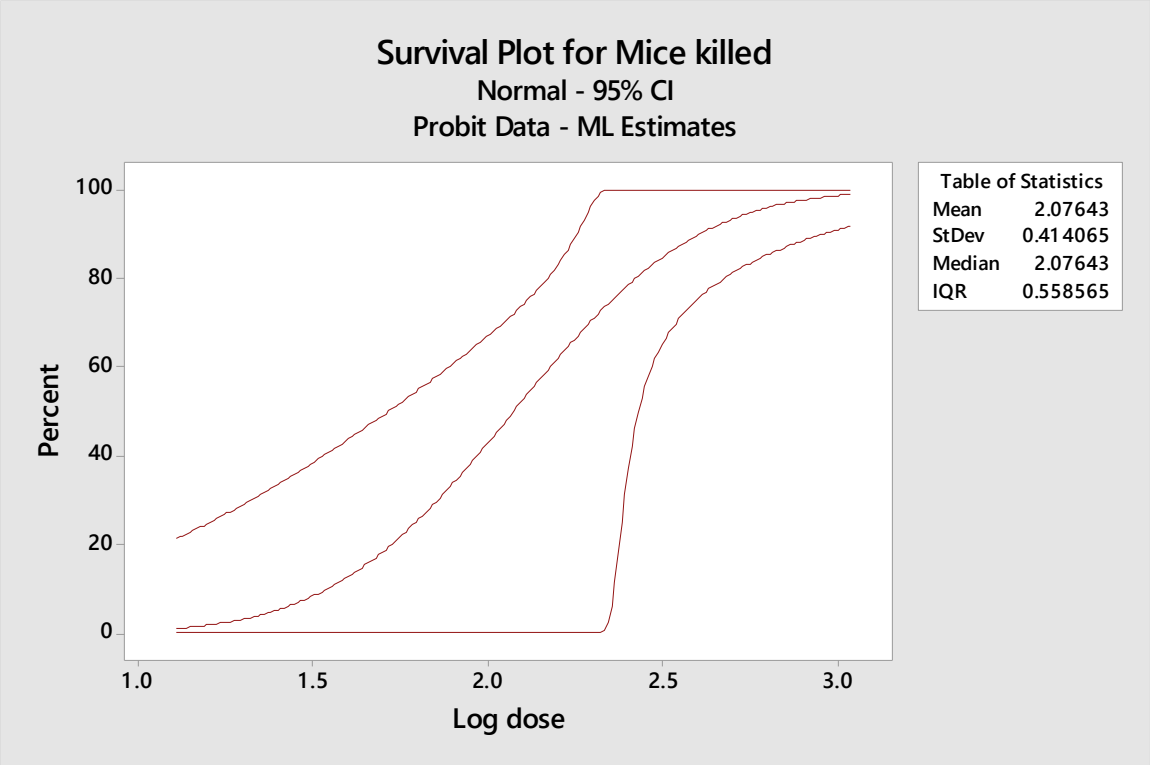
Parameter	Estimate	Standard Error	95.0% Normal CI Lower	Upper
Mean	2.07643	0.182217	1.71929	2.43357
StDev	0.414065	0.205962	0.156196	1.09766

Table of Percentiles

Percent	Percentile	Standard Error	95.0% Fiducial CI Lower	Upper
1	3.03969	0.366057	2.65457	30.0181
2	2.92681	0.313748	2.58909	25.5270
3	2.85520	0.281327	2.54564	22.6794
4	2.80133	0.257496	2.51148	20.5387
5	2.75751	0.238577	2.48238	18.7988
6	2.72021	0.222888	2.45635	17.3191
7	2.68750	0.209517	2.43226	16.0229
8	2.65822	0.197914	2.40938	14.8637
9	2.63159	0.187720	2.38718	13.8108
10	2.60707	0.178690	2.36520	12.8432
20	2.42491	0.129077	2.04343	5.81131
30	2.29356	0.125854	-0.228330	2.78056

40	2.18133	0.147991	-4.41725	2.43869
50	2.07643	0.182217	-8.53249	2.31910
60	1.97153	0.223496	-12.6840	2.23580
70	1.85929	0.271842	-17.1399	2.16088
80	1.72794	0.331422	-22.3630	2.08147
90	1.54578	0.416913	-29.6135	1.97832
91	1.52127	0.428578	-30.5896	1.96480
92	1.49464	0.441284	-31.6500	1.95019
93	1.46536	0.455289	-32.8162	1.93420
94	1.43265	0.470972	-34.1186	1.91644
95	1.39535	0.488904	-35.6042	1.89627
96	1.35153	0.510030	-37.3497	1.87271
97	1.29766	0.536074	-39.4957	1.84389
98	1.22604	0.570804	-42.3486	1.80581
99	1.11317	0.625745	-46.8457	1.74623





APPENDIX 5: *Cyperus articulatus* probit analysis

Probit Analysis: Mice killed, Number of mice versus Log dose
Distribution: Normal
Response Information

Variable	Value	Count
Mice killed	Event	15
	Non-event	10
Number of mice	Total	25

Estimation Method: Maximum Likelihood
Regression Table

Variable	Coef	Standard Error	Z	P
Constant	24.9009	10.0303	2.48	0.013
Log dose	-9.83017	3.93174	-2.50	0.012
Natural Response	0			

Log-Likelihood = -7.405
Goodness-of-Fit Tests

Method	Chi-Square	DF	P

Pearson	1.10458	3	0.776
Deviance	1.34879	3	0.718

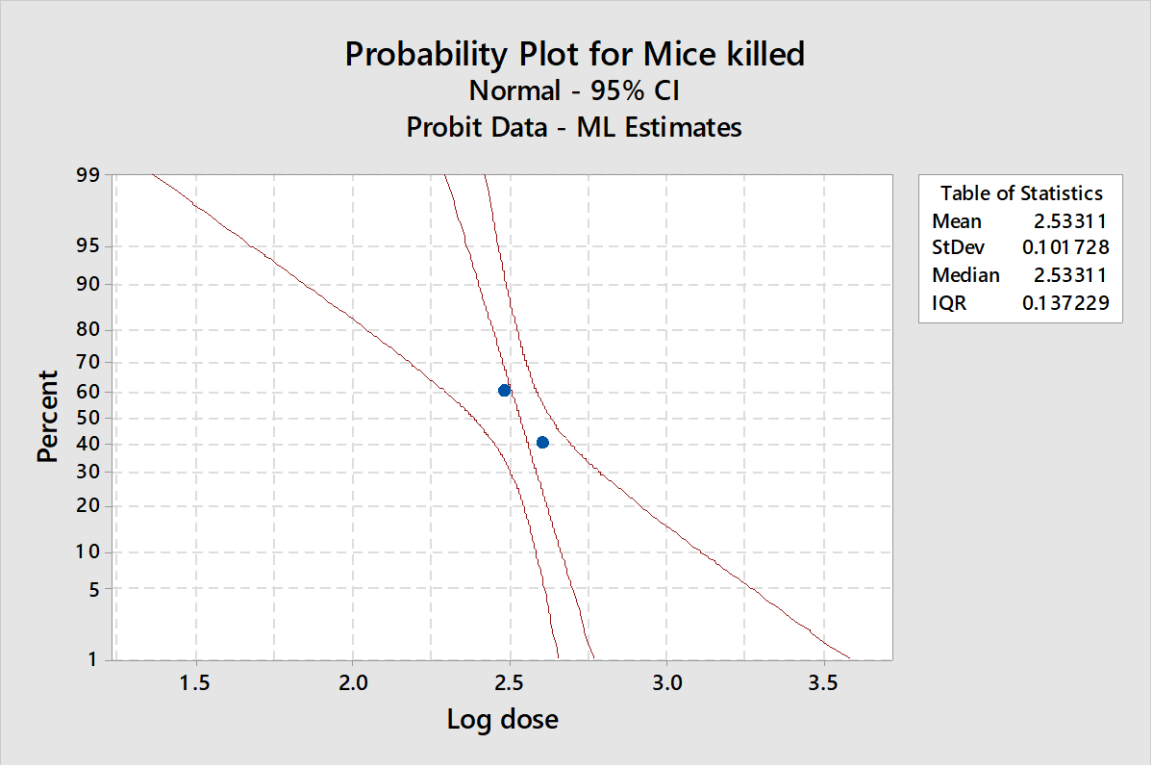
Tolerance Distribution
Parameter Estimates

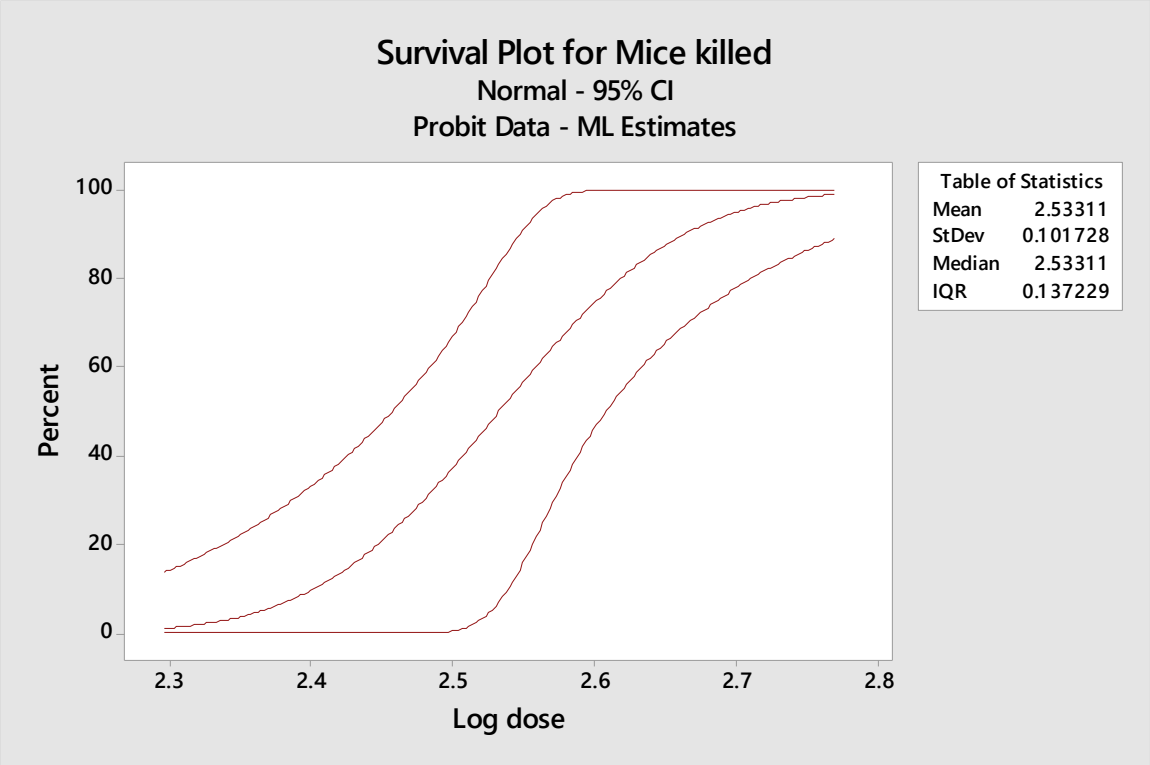
Parameter	Estimate	Standard Error	95.0% Normal CI Lower	Upper
Mean	2.53311	0.0384345	2.45778	2.60844
StDev	0.101728	0.0406876	0.0464502	0.222788

Table of Percentiles

Percent	Percentile	Standard Error	95.0% Fiducial CI Lower	Upper
1	2.76976	0.0959481	2.65723	3.58504
2	2.74203	0.0858694	2.63952	3.45887
3	2.72444	0.0796145	2.62796	3.37915
4	2.71120	0.0750007	2.61903	3.31940
5	2.70044	0.0713174	2.61160	3.27098
6	2.69127	0.0682398	2.60512	3.22991
7	2.68324	0.0655911	2.59930	3.19404
8	2.67604	0.0632639	2.59397	3.16204
9	2.66950	0.0611880	2.58901	3.13305
10	2.66348	0.0593151	2.58433	3.10648
20	2.61872	0.0469539	2.54438	2.91424
30	2.58645	0.0406828	2.50420	2.78698
40	2.55888	0.0380718	2.45292	2.69520
50	2.53311	0.0384345	2.38313	2.63127
60	2.50734	0.0414424	2.29336	2.58732
70	2.47976	0.0470089	2.18352	2.55410
80	2.44749	0.0556327	2.04589	2.52429
90	2.40274	0.0698140	1.84773	2.49026
91	2.39672	0.0718493	1.82070	2.48603
92	2.39017	0.0740858	1.79128	2.48151
93	2.38298	0.0765727	1.75886	2.47660
94	2.37494	0.0793815	1.72257	2.47120

95	2.36578	0.0826209	1.68109	2.46514
96	2.35501	0.0864703	1.63226	2.45811
97	2.34178	0.0912585	1.57209	2.44961
98	2.32419	0.0977044	1.49192	2.43849
99	2.29645	0.108014	1.36522	2.42131





APPENDIX 6: *Rhynchosia usambarensis* probit analysis

Probit Analysis: Mice killed, Number of mice versus Log dose
Distribution: Normal
Response Information

Variable	Value	Count
Mice killed	Event	9
	Non-event	16
Number of mice	Total	25

Estimation Method: Maximum Likelihood
Regression Table

Variable	Coef	Standard Error	Z	P
Constant	2.08980	2.52963	0.83	0.409
Log dose	-1.01716	1.04664	-0.97	0.331
Natural Response	0			

Log-Likelihood = -15.856
Goodness-of-Fit Tests

Method	Chi-Square	DF	P

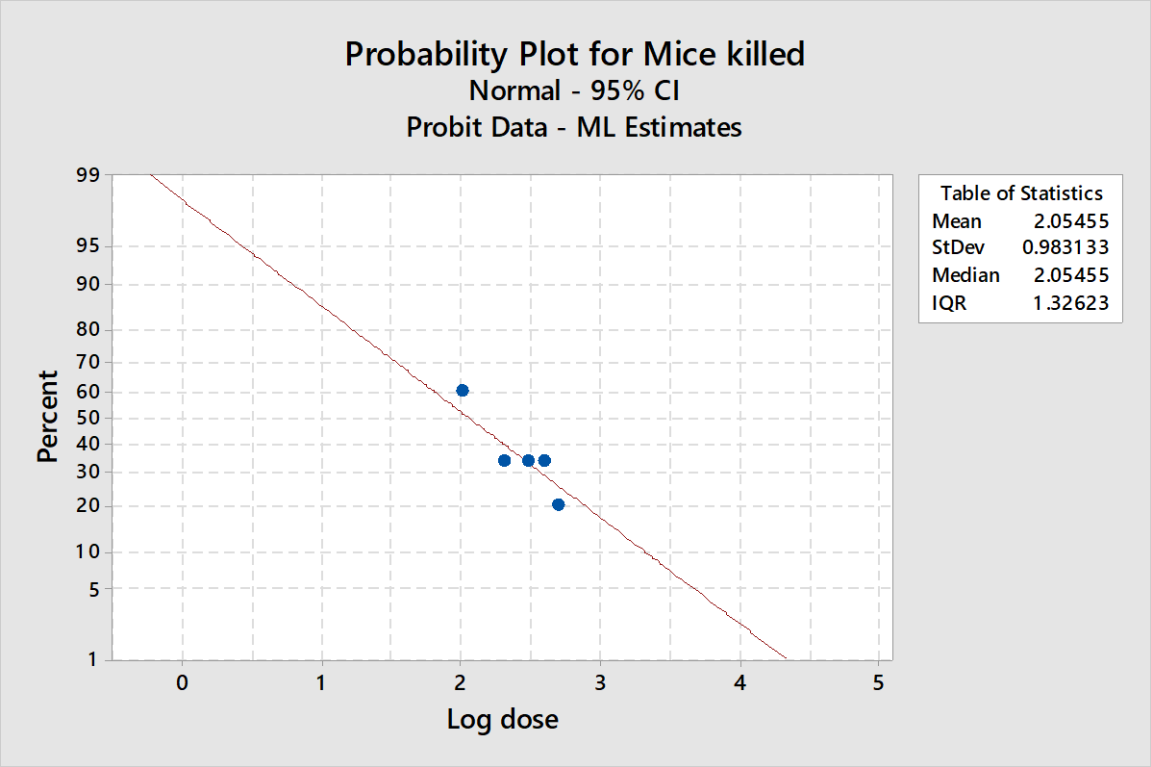
Pearson	1.44647	3	0.695
Deviance	1.51404	3	0.679

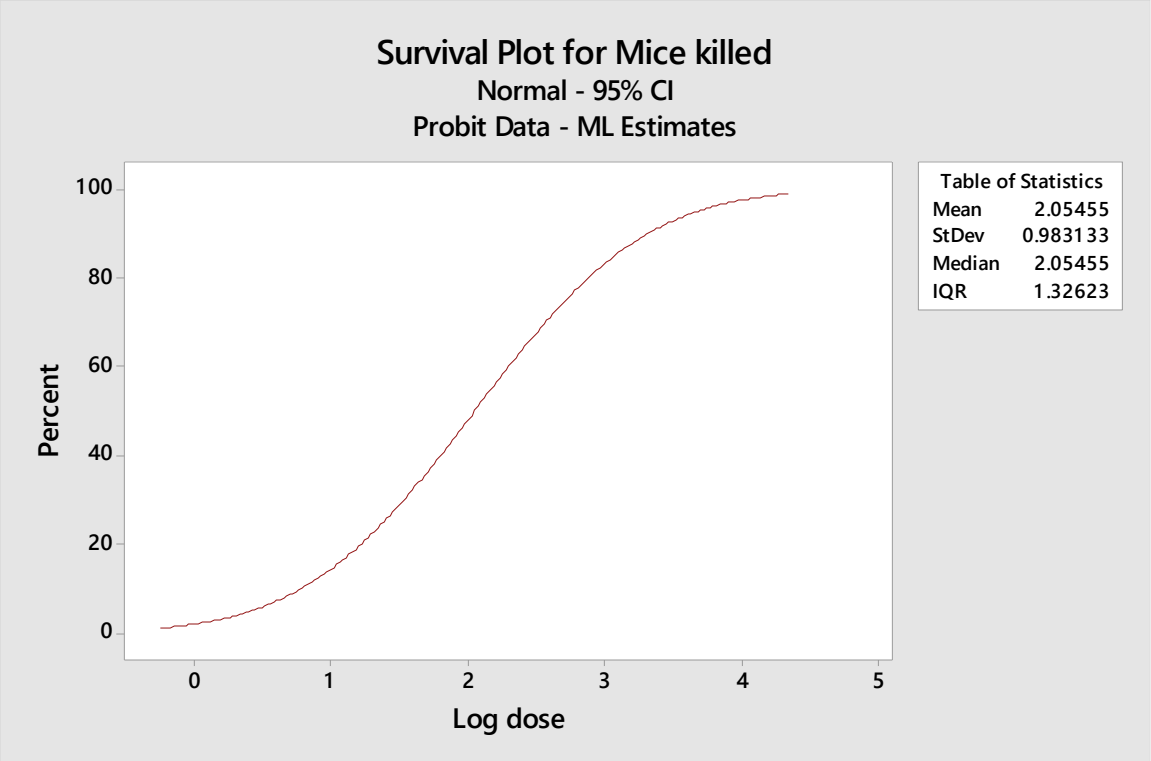
Tolerance Distribution
Parameter Estimates

Parameter	Estimate	Standard Error	95.0% Normal CI Lower	Upper
Mean	2.05455	0.441137	1.18994	2.91916
StDev	0.983133	1.01163	0.130839	7.38730

Table of Percentiles

Percent	Percentile	Standard Error	95.0% Fiducial CI Lower	Upper
1	4.34166	2.00997	*	*
2	4.07366	1.73679	*	*
3	3.90362	1.56394	*	*
4	3.77571	1.43424	*	*
5	3.67166	1.32903	*	*
6	3.58310	1.23972	*	*
7	3.50545	1.16165	*	*
8	3.43592	1.09197	*	*
9	3.37269	1.02882	*	*
10	3.31449	0.970921	*	*
20	2.88198	0.554048	*	*
30	2.57011	0.307203	*	*
40	2.30362	0.275504	*	*
50	2.05455	0.441137	*	*
60	1.80548	0.666838	*	*
70	1.53900	0.926114	*	*
80	1.22713	1.23775	*	*
90	0.794616	1.67574	*	*
91	0.736411	1.73496	*	*
92	0.673179	1.79934	*	*
93	0.603653	1.87018	*	*
94	0.526002	1.94937	*	*
95	0.437442	2.03974	*	*
96	0.333394	2.14601	*	*
97	0.205481	2.27674	*	*
98	0.0354433	2.45069	*	*
99	-0.232558	2.72511	*	*


Probability Plot for Mice killed
Normal - 95% CI
Probit Data - ML Estimates
Percent
99
95
90
80
70
60
50
40
30
20
10
5
1
0
1
2
3
4
5
Log dose
Table of Statistics
Mean 2.05455
StDev 0.983133
Median 2.05455
IQR 1.32623


Survival Plot for Mice killed
Normal - 95% CI
Probit Data - ML Estimates
Percent
100
80
60
40
20
0
0
1
2
3
4
5
Log dose
Table of Statistics
Mean 2.05455
StDev 0.983133
Median 2.05455
IQR 1.32623