2017-12

Evaluation of tick repellency activity and toxicity of commiphora swynnertonii (burtt) exudates

Swai, Disela Edwin

NM-AIST

EVALUATION OF TICK REPELLENCY ACTIVITY AND TOXICITY OF *Commiphora swynnertonii* (BURTT) EXUDATES

Disela Edwin Swai

A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Master's in Life Science of the Nelson Mandela African Institution of Science and Technology

Arusha, Tanzania

December, 2017

ABSTRACT

This thesis reports the repellency activity and toxicity of *Commiphora swynnertonii* exudates. The exudates were extracted using hexane and chloroform solvents. The crude extracts were tested for repellency activity at concentration of 110, 100, 90, 80, 70, 60 and 50 mg/ml against *Rhipicephalus appendiculatus* larvae. The crude extracts were further evaluated for the acute and subacute toxicity using albino mice and rats, respectively. The results showed that repellency activity appeared to be concentration and time dependent. In acute toxicity, oral administration of *C. swynnertonii* hexane and chloroform extract at a doses of 500, 1000, and 2000 mg/kg body weight induced no clinical signs of toxicity in the mice during 14 days of experimental period. However, *C. swynnertonii* chloroform extract showed some toxic properties in some of blood parameters and in vital organs (liver and kidney) of treated mice. In subacute toxicity, *C. swynnertonii* hexane extract showed significant change in some of hematological and biochemical parameters. Even though rats treated with *C. swynnertonii* chloroform extract did not develop any observable sign of toxicity, some toxic properties was observed in some of blood parameters and in vital organs (liver and kidney). It is concluded that, *C. swynnertonii* hexane and chloroform extracts considered as good repellency agents against *Rhipicephalus appendiculatus* larvae. For the toxicity evaluation, *C. swynnertonii* hexane extracts was nontoxic while *C. swynnertonii* chloroform extract had toxic properties deduced from change in blood parameters and internal organs of treated animals.

DECLARATION

I, **Disela Edwin Swai** do hereby declare to the Senate of Nelson Mandela African Institution of Science and Technology that this thesis is my own original work and that it has neither been submitted nor being concurrently submitted for degree award in any other institution.

…… …….

Disela Edwin Swai **Date**

Name and signature of candidate

COPYRIGHT

CERTIFICATION

The undersigned certify that they have read and hereby recommend for examination of a thesis entitled **"Evaluation of Tick Repellency Activity and Toxicity of *Commiphora swynnertonii* (Burtt) exudates"** in fulfillment of the requirements for the Degree of Master in Life Sciences at Nelson Mandela African Institution of Science and Technology (NM-AIST).

…… ……..

Dr. Musa Chacha **Date**

1st Supervisor

…….. ……..

Dr. Paul Erasto **Date**

2nd Supervisor

AKNOWLEDGEMENT

First and foremost, I would like to thank my Almighty God for his enormous grace, blessing and keeping me healthier during the whole period of this study.

I would like to express my special gratitude to my supervisors, Dr. Musa Chacha and Dr. Paul Erasto for their diligent guidance, assistance, invaluable advice and encouragement throughout the period of this study. Their support, concern and fidelity have contributed greatly to the success of my study.

Special thanks to RUFORUM through RUFORUM CAA Project for funding my study at NM-AIST. My heartfelt thanks go to Dr. Musa Chacha, the project investigator of RUFORUM CAA Project for entrusting and offering me the scholarship.

I would like to thank my fellow student Mr. Sylvester Temba whom we helped each other during some field and laboratory work. Special thanks go to Mr. Muhuji Kilonzo and Okumu Ochanga, PhD students for their help and encouragement towards the success of this study. I would like also to express my sincerely thanks to laboratory technician from Sokoine University of Agriculture (SUA) Mr. Mkuchu, Mr. Alfred Mwinyika, Mr. Ramadhan and Mr. Mlai for their strong cooperation in carrying out hematological, biochemical and Histopathological analysis during toxicity study. I would like also to extend my sincerely appreciation to Mr. John Mshanga, laboratory technician from NM-AIST for his cooperation during toxicity study.

Enormously, I thank my husband Mr. Bernard Mawala for his support and encouragement throughout the period of my study. He has been a constant source of support and encouragement during the challenges of my studies. I am also very thankful to my brilliant daughter for her patience throughout my study period because most of the time I was not so close to her during this period. I extend my deeper appreciation and gratitude to my young sister Fderka for her patience, encouragement and taking care to my daughter during the whole period of my study.

DEDICATION

I would like to dedicate this thesis to my beloved husband (Bernard Mawala) for his patience, understanding, advice, encouragement, prayer and moral support, my daughter (Viviane Bernard) for her patience, understanding and prayer and mostly my parents (Edwin Swai and Theresia Joseph) for their prayer and encouragement throughout the whole period of my study

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABRIVIATION

ALP	-	Alkaline Phosphate
ALT	-	Alanine Aminotransferase
ANOVA	-	Analysis of Variance
AST	-	Aspartate Aminotransferase
BHC	-	Benzenehexachloride
BW	-	Body Weight
DDT	-	Dichlorodiphenyltrichloroethane
DEET	-	N, N-Diethyl-meta-toluamide
DMSO	-	Dimethyl sulfoxide
DPPH	-	1, 1-diphenyl-2-picryl hydrazyl
DRC	-	Democratic Republic of Congo
EC_{50}	-	Effective concentration
ECF	-	East Coast Fever
EDTA	-	Ethylenediaminetetraacetic acid
FAO	-	Food and Agriculture Organization
FXR	-	Farnesoid X Receptor
GCC	-	Gravitational column chromatography
Hb	-	Hemoglobin
HCT	-	Hematocrit
HDL	-	High Density Lipoprotein Cholesterol
IC_{50}	-	Inihibition Concentration
LD_{50}	-	Lethal dose
LDL	-	Low-Density Lipoprotein
LYM	-	Lymphocyte
MCH	-	Mean hemoglobin concentration
MCHC	-	Mean corpuscular hemoglobin concentration
MCV	-	Mean Corpuscular Volume
ML	-	Macrocyclic Lactone
MON	-	Monocytes
MPV	-	Mean Platelet Volume
NEU	-	Neutrophil
NIMR	-	National Institute for Medical Research

NTF	-	Number of ticks on non-impregnated filter paper
OECD	-	Organization for Economic Co-operation and Development
OP	-	Organophosphates
PMNs	-	Polymorphonuclear cells
PR	-	Percentage Repellent
RBC	-	Red blood cell
RDW	-	Red blood cell distribution width
SEM	-	Standard error of the mean
ML	-	Macrocyclic Lactone
BHC	-	Benzenehexachloride
TBDs	-	Tick-borne diseases
TF	-	Number of ticks on impregnated filter paper
TLC	-	Thin Layer Chromatography
TPRI	-	Tanzania Pesticides Research Institute
UV	-	Ultraviolet
VLDL	-	Very Low-Density Lipoprotein
WBC	-	White Blood Cell

CHAPTER ONE

INTRODUCTION

1.1 Background information

The biggest challenge to livestock industries among pastoralist especially in African continent is tick infestation and tick borne diseases (Mkangara *et al.*, 2015; Nyabayo *et al.*, 2015). In sub-Saharan Africa, East coast fever (ECF) caused by *Theileria parva*, transmitted by brown ear tick, *Rhipicephalus appendiculatus* is one of the major constraints to the development of livestock industry (Wanzala *et al*., 2014). Ticks infestation and tick borne diseases have great negative impacts on economics to livestock keepers (Kalala *et al*., 2014). In east Africa, the loss due to ECF was estimated to be 168 million United States Dollar including a mortality of 1.1 million cattle (Mukhebi *et al*., 1992). It was also reported that in 1981-1993 tick-borne diseases accounted for 71.4% of annual cattle death in Tanzania. However, in 2006, Kivaria reported that, cattle death due to ECF in Tanzania was estimated to be 68% which attributed to the loss of 364 million USD (Kivaria, 2006). In Uganda the cost incurred for the control of ticks and tick-borne diseases was 308 144 USD (Ocaido *et al*., 2009).

The use of synthetic acaricides is the most common method used for decades by livestock keepers to control both hard and soft ticks (Opdebeeck *et al*., 1988). Synthetic acaricides that has been employed for the management of ticks are organophosphates (OP), phyrethroid, amitraz, macrocyclic lactone (ML), arsenic, organochlorine, benzenehexachloride (BHC), polychloroterpine, dieldrin and aldrin, cyclodiene compounds and toxaphene just to mention a few. Despite the fact that synthetic acaricides has been effective in the management of ticks, their use has become less reliable, unacceptable and unsustainable due to several reasons, such as high cost of acaricides, tick resistance to synthetic acaricides and contamination of the environment or food with toxic residues (Bissinger *et al*., 2010; Kalala *et al*., 2014; Mkolo *et al*., 2007).

Development of tick resistance to a successive acaricides in recent years has been a major problem in livestock farmers (Sindhu *et al*., 2003; Kivaria *et al*., 2007). Baker (1978) reported resistance of ticks to Arsenical acaricides in *Boophilus decoloratus* which was first detected in South Africa in 1941 and resistance to toxaphene have been reported from Kenya, Malawi, South Africa, Uganda, Zambia and Zimbabwe (FAO, 1984). *Boophilus microplus* resistance to Arsenical was also reported in Australia in the year 1937 (George *et al*., 2004). In 1971, Brown and Pal reported that the resistance of *B. microplus* to chlorinated cyclodiene, dichlorodiphenyltrichloroethane (DDT) and organophosphorus / Carbamate acaricides was first observed in 1950, 1954 and 1964, respectively, and from this time resistance has been reported with all acaricides used and for most tick species throughout the world (FAO, 1984). These challenges prompted the necessity to seek for alternative acaricidal agents which are affordable, effective, eco-friendly and less toxic to the livestock and livestock keepers.

Exploitation of naturally occurring products has been promoted as an alternative way of addressing the problems associated with the use of synthetic drugs (Opiro *et al.,* 2013). The use of plant-based products to kill or repel parasitic arthropods on livestock has been widely used by many communities as an alternative to synthetic acaricides (Opiro *et al.*, 2013). For example in Tanzania and Kenya, the Maasai tribe use sap of *Commiphora swynnertonii* and applied on animal skins for the management of ticks, fleas and tsetse flies (Kalala *et al*., 2014). Different studies have been done to validate the ethnoveterinary use of plant - based product as an alternative to synthetic drugs. For instant, a study conducted by Lima *et al*. (2016) on repellency activity of *Lippie alba* essential oils grown in Brazil found that the plant possessed repellency activity against *Rhipicephalus appendiculatus.* On another study, Wanzala *et al*. (2014) observed repellency activity of *Tagetes minuta* and *Tithonia diversifolia* grown in Kenya against *R. appendiculatus.* Another study done by Nyabayo *et al*. (2015) on acaricidal activity of *Salvia nilotica* essential oils against *R. appendiculatus* reported that the essential oils of *Salvia nilotica* exhibited acaricidal activity against *R. appendiculatus.*

Commiphora (Burseraceae) is among plants claimed to have wide-range of biological activities. In Tanzania, *Commiphora* species is demanded in the treatment of wounds, cough, chest aliments in human and control of ecto parasites (Kaoneka *et al*., 2002). *Commiphora swynnertonii* is a small highly branched and thorny tree with a height of 3 meters tall. This plant is readily available in the Northern part of Tanzania (Ruffo *et al*., 2002), Southern part of Kenya (Kalala *et al*., 2014), tropical and subtropical part of Asia and South America

(Mkangara *et al*., 2015). Since *Rhipicephullus appendiculatus*, *Amblyomma variegatum*, *Amblyomma gemma*, *Amblyomma lepidum*, *Boophillus microplus* and *Rhipicephullus averts* are ticks of economic importance in Tanzania (Kalala *et al*., 2014; FAO, 1983), repellent agent from *Commiphora swynnertonii* exudates contain volatile compound will be established, which will comfort livestock to stay all the time without tick infestation as those ticks will be repelled with the compound found in the repellent agent. Additionally, toxicity of *C. swynnertonii* extracts will be evaluated.

1.2 Problem statement

Tick infestations and tick-borne infections is the common veterinary health problem in Tanzania. For instance, ECF alone was estimated to account for 68% of the 364 million USD annual total losses resulting from tick-borne diseases in Tanzania (Kivaria, 2006). The strategy to lower losses of cattle caused by tick infestation in Tanzania has been through the use of synthetic acaricides usually suspended to water and applied on the animal skins to prevent tick manifestation. Despite of promising results through the use of synthetic acaricides, development of tick resistance to some synthetic acaricides has been reported in many parts of the country (Higa *et al*., 2015). Non-biodegradability, bioaccumulation, and bio magnifications of some synthetic acaricides are not favored by environmentalists since they negatively affect ecosystem including human beings. High cost associated with the use of synthetic acaricides and the aforementioned reasons, hinders the development of livestock industry and seriously affect small holder farmers in Tanzania. Thus, the development of affordable and eco-friendly repellent agent is of paramount importance. Repellency activity and toxicity of *C. swynnertonii* extracts will validate the potential use of *C. swynnertonii* exudates.

1.3 Research objectives

1.3.1 General objective

To establish repellency efficacy and evaluate toxicity of *C. swynnertonii* exudates

1.3.2 Specific objectives

(i) To evaluate repellency activity of hexane and chloroform extracts from *C. swynnertonii* exudates

(ii) To evaluate toxicity of hexane and chloroform extract using albino mice and rats

1.4 Significant of the study

The study evaluated the repellency effect of *C. swynnertonii* exudates. Furthermore, the toxicity study provided the safety margin of the extracts from *C. swynnertonii* exudates.

CHAPTER TWO

LITERATURE REVIEW

2.1 The genus Commiphora

The genus *Commiphora* (Burseraceae) encompasses approximately 190 species of shrubs and trees and are distributed throughout the sub-tropical region of Africa, the Western and Eastern Indian Ocean islands, and Arabia (Daly *et al*., 2001), China, Egypt, northern Namibia, Iran, Pakistan, Peninsula, Sri Lanka and Brazil (Soromessa, 2013). In Tanzania, *Commiphora* species have different local names according to tribes: mbambara, mponda, mturituri, mtwitwi, (Swahili); oltemwai (Maasai); mguta (Sukuma); dumbechanda (Taturu); mzilanzi (Gogo) (Minja 1999; Sambuta and Masola, 2008). *Commiphora* species produces resins and gums which have been known by mankind since ancient time. The frankincense, incense and myrrh are of biblical fame. The genus name *Commiphora* originates from the Greek words Kommi meaning 'gum' and pheros meaning 'bearing' (Van der Walt, 1974). The Afrikaans name for *Commiphora* is 'kanniedood', means 'cannot die'. This is an indication of sustainability of the plant and also refers to the fact that the truncheons grow easily when planted.

Commiphora species are characterized by small trees or shrubs range from 3.5 – 4.0 m tall (Paraskeva *et al*., 2008) with spinescent branches, pale grey bark and reddish-brown resinous exudates (Suleiman, 2014). *Commiphora swynnertonii* is a small tree highly branched with spine reaching a height of about 3 meters (Kalala *et al*., 2014). The barks are pale grey that peels off in papery pieces. When damaged the bark release a watery, milky sap and later become reddish brown resinous exudates. Leaves vary between simple and compound leaves, shiny copper green and during wet season the leaves arise and detached during dry season.

Commiphora swynnertonii was first collected by Burtt, B.D. in Dodoma District, Tanzania in 1932 and kept at National Museums of Kenya, East Africa Herbarium, Nairobi Kenya with voucher specimen no 3827 (Bakari, 2013). Products of *Commiphora* species have been used since biblical fame. Moses used myrrh during sacred Jewish ceremonies as anointing oil and it was one of the three gift given to infant Jesus ('the present unto him a gifts, gold and frankincense') and the oil from myrrh was used to anoint the body of Christ after crucifixion (Drarmananda, 2003). Myrrh from *Commiphora* species has been used as medicine for treatment of both livestock and human diseases. The myrrh was used for the treatment of menstrual pain in women thus considered as an agent that promotes the inception of

menstruation and regulates its flow (Hanus *et al*., 2005). The sap of *C. swynnertonii* has for long time been utilized as anti-ectoparacites and is applied on animals for control of ticks, fleas, tsetse flies, bed bugs and mange mites (Minja, 1999; Bakari, 2013).

2.1.1 Phytochemical investigations of *Commiphora* species

Plants have ability to synthesize secondary metabolites, which are used as bio-resource in food supplements, and chemical entities for synthesis of drugs in medicine (Cowan, 1999). In many cases, secondary metabolites produced by plants serve as plant defense mechanisms against predation by microorganisms, insects, and herbivores. Most of these secondary metabolites have been found in different *Commiphora* species and their concentrations vary widely depending on species, season and geographical location. Bioactive secondary metabolites which have been investigated from *Commiphora* species are Dammarine triterpenes, Ferulate, Furanosesquiterpenes, Guggultetrols, guggulsterones, Lignans, Flavonones, Sesquiterpenes, esters, Cumunic aldehyde Euginol, Steroids, resin acids and Protein (Zhu *et al*., 2001; Fatope *et al*., 2003; Al-Harbi *et al*., 1997). Phytochemical screening of *C. swynnertonii* revealed presence of Saponins, Flavonoids, Cardiac glycosides, Terpenoids, Tannins and Steroids (Bakari, 2013). Phenolic compounds, tannins, alkaloids, triterpenes and sterols have been reported from ethanolic and hexane extracts of *C. africana* (Hanus *et al*., 2005; Aliyu *et al*., 2007). Leaves of *C. swynnertonii* were reported to contain essential oils which were five sesquiterpenoids and four sesquiterpenoid derivatives, copaene and isocaryophyllene (Kaoneka *et al*., 2007). Isolated secondary metabolites from some of *Commiphora* species have been reported to exihibit *in vitro* and *in vivo* pharmacological activities such as antiproliferative, antioxidant, anti-inflammatory and antimicrobial (Bakari *et al*., 2011). Most of phytochemical studies on the genus *Commiphora* have been concentrated on the myrrh oil extracted from the stem bark because of the exploitation of the oil in modern perfume formulation and also used as an astringent for the mucous membranes of the mouth and throat (Hanus *et al*., 2005).

2.1.2 Uses of Commiphora species

Some *Commiphora* species have antiectoparasitic, antiendoparasitic and repellency effects. Example *C. swynnertonii* and *C. holtziana* have been reported to possess larvicidal, and acaricidal activity against cattle ticks *R. appendiculatus, Amblyomma variegatum* and *Boophilus microplus* (Sambuta and Masola, 2006; Mkangara *et al*., 2015; Birkett *et al*., 2008). Carroll *et al*. (1989) reported larvicidal and repellency activity of hexane extract of the

gum of *C. erythraea* (Engler) against *Amblyomma americanum* and *Dermacentor variabilis*. *Commiphora erythraea* and *C. incise* have been reported to be used by Borana and Gerri people of Southern Ethiopia for the control of ectoparasites (Bekalo *et al*., 1996). Myrrh from *C. molmol* has been reported to be effective in the treatment of human schistosomiasis and fasciolosis (Massoud *et al*., 2001; Haridy *et al*., 2004). *Commiphora molmol* was also evaluated in treating sheep which are naturally infected with *Moniezia expansa* and showed a cure rate of 100% with no observable clinical side effects in the sheep (Al-Mathal and Fouad, 2004).

Several *Commiphora* species have been reported to have activities in reducing serum cholesterol concentrations without causing any detrimental side effects (Adebayo *et al*., 2006). *Commiphora mukul* has been claimed to lower serum cholesterol and triglycerides as well as low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL) (the "bad" cholesterols) and at the same time increasing high density lipoprotein cholesterol (HDL) (Singh *et al*., 1994). Guggulsterone, a compound found in *C. mukul* exert activity in lowering serum cholesterol by acting as an antagonist of the farnesoid X receptor (FXR), a bile acid receptor which is important in cholesterol metabolism (Urizar *et al*., 2002; Huang *et al*., 2003). *Commiphora africana* and *C. myrrha* extracts were also reported to exhibit hypolipidaemic activity in experimental rats (Adebayo *et al*., 2006).

An ethanolic stem bark extract of *C. africana* was reported to have anti-hyperglycemic potential which was associated with stimulation of the pancreatic beta-cells in alloxan induced-diabetic rats (Goji *et al*., 2009). However, *C. mukul* extracts was shown to have beneficial effect in the treatment of diabetes by decreasing the effect of blood glucose and increasing levels of plasma insulin in Streptozotocin induced diabetic rats (Bellakonda *et al*., 2011). Anti-ulcerative effect of *C. opobalsamum* against different acute gastric ulcer models in rats induced by necrotizing agents was investigated by Al-Howiriny *et al*. (2005). The results showed that the extract had a dose-dependent protection against ethanol-induced depletion of gastrointestinal mucosa through supporting both the offensive and defensive factors (Al- Howiriny *et al*., 2005). Similarly, protection of production of stomach mucus by the extract of *C. molmol* (oleo-gum resin) was reported by Al- Harbi *et al*. (1997).

Antimicrobial activities of different species of genus *Commiphora* have been reported by different scientific researchers. Example All *et al*. (2007) reported the antibacterial activity of toluene-methanol extracts from the leaves and roots of *C. quadricincta* against *Yersinia*

enterocolitica, Staphylococcus aureus, *Escherichia coli* and *Staphylococcus epidermidis*. *Commiphora africana* root ethanolic extract was investigated to have antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Salmonella typhi* (typhoid) and *Candida albicans* (Paraskeva *et al*., 2008; Akor and Anjorin, 2009). Antimicrobial activities of *C. kerstingii* against *Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Pseudomonas fluorescens* and *Bacillus subtilis* was reported by Musa (2008). The resin from *C. mukul* has been investigated by Newton *et al*. (2002) to exihibit antimycobacterial activity, against *Mycobacterium aurum.*

Several *Commiphora* species have been documented to have anti-inflammatory and wound healing effect. *In vitro* anti-inflammatory activity of *C. pyracanthoides, C. schimperi* and *C. glandulosa* extracts were reported by Paraskeva and his coworker (2008). Anti- inflammatory effect of *C. africana* stem bark ethanolic extracts in adult Wistar rats using paw edema model was reported by Ezekiel *et al*. (2010). Myrrh from *C. molmol* was found to have wound healing properties in wounded rats by complete epithelialization, new granulation tissues formation and new blood vessels development, leucocytes infiltration with increased polymorphonuclear cells (PMNs) (Haffor, 2010).

Antioxidant agents of medicinal plants are possible mediators in the protection against cancers, myocardial necrosis and increased fibrinolysis. Some *Commiphora* species have been reported to have antioxidant effects. Example methanolic extracts of *C. berryi* and *C. caudata* have been reported to display significant 1, 1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity, with IC_{50} values of 26.9 and 21.2µg/mL respectively (Kumari *et al*., 2011).

2.2 Toxicity of Commiphora species

Plant products are assumed to be nontoxic and less destructive to the human body than convectional drugs because they are of natural origin (Nasri and Shirzad, 2013). Some plant products which are being used for treatment of different illnesses are reported to be poisonous which may resulted into allergic reaction in people who using the products and may cause damages to the internal organs such as liver, heart, lungs, spleen and kidneys (Moreira *et al*., 2014). Toxic effects are being attributed with several factors including over dosage, lack of standardization and incorrect preparations (Goldman, 2001). In evaluating toxicity of drugs or plant based products, preliminary toxicity based on brine shrimp lethality bioassay may be applied which could provide an indication of toxic profile of the tested materials (Meyer *et*

al., 1982; Carballo *et al*., 2002). Studies have been done to evaluate cytotoxicity study of some *Commiphora* species using brine shrimp lethality test so as to draw inferences on the safety of such plants. For example Mkangara *et al*. (2015) evaluated the cytotoxicity of chloroform, ethyl acetate and methanol extracts from *C. swynnertonii* in brine shrimps larvae.

Toxicity using animal model, depending on the duration of exposure of the animal to drugs or plants substances may be of three studies namely acute toxicity study, subacute toxicity study and chronic toxicity study (Baki *et al*., 2007). Acute toxicity indicates the adverse effects occurring within a short period after oral administration of a single dose of the tested substance in which the animal should be observed daily for a period of 14 days (Duffus *et al*., 2009). Subacute toxicity studies, the animals are administered repeated doses of drug and observation to each individual animal is done daily for a period of 28 days (Baki *et al*., 2007). Similarly, the chronic toxicity is the adverse effects occurring when the experimental animals are exposed to daily repeated doses of drug for a period of 90 days (Belguet, 2010). Several studies have been conducted on acute, subacute and chronic toxicity of some *Commiphora* species using animal model. For example acute and chronic toxicity study of *C. molmol* (oleo–gum–resin) in mice was reported by Rao and his coworkers (2001) the results showed that *C. molmol* (oleo–gum–resin) was considered to be safe in human consumption. Another study was conducted by Mekonnen *et al*. (2003) who evaluated the acute toxicity of *C. myrrha, C. guidottii* and *C. erlangeriana,* in Swiss albino mice. The results showed that non polar extracts of *C. myrrha* and *C. guidottii* did not induce any toxic at a dose of 1.2g/kg and 0.96g/kg. However the results showed that the extract of *C. erlangeriana* had a mean lethal dose (LD_{50}) value of 410mg/kg body weight (Mekonnen *et al*., 2003).

2.3 Tick infestation and tick-borne diseases

Ticks are ectoparasites which are the main vectors for disease causing agents to humans, livestock and wild animals all over the world (Aydin *et al*., 2015). Ticks be- long to phylum arthropoda, order acarina and are divided into soft bodied ticks (Argasidae) and hard bodied species (Ixodidae) (Iqbal *et al*., 2006). The family Ixodidae, hard ticks contains about 683 species while Argasidae family, soft ticks contains 185 species (Jongejan and Uilenberg, 2004; Latif and Walker, 2004). Ticks are the most important ecto-parasites of livestock in tropical and sub-tropical areas, and are responsible for severe economic losses in livestock (Iqbal *et al*., 2006).

The life cycles of ticks involve four stages such as egg, larva, nymph and adult (Horak *et al*., 2002). Eggs are deposited in grassy areas or woods. After hatching the small, 6-legged larvae usually feed on small hosts such as rodents, rabbits, and squirrels. After initial feeding of the larvae, it molt into 8-legged nymphs which are approximately 2/3 the size of adults. Nymphs and adults usually feed on larger hosts such as deer, dogs, and humans. During tick life cycle, they display different progressive patterns and are grouped as one-, two- and three-host ticks. In one-host ticks all stages are found on the same host and only the gravid female drop off to the environment where she lays eggs and then dies. Two-host ticks larvae and nymphs feed on the same host and engorged nymphs drop off and molt in the environment into adults where they seek a new host. Three-host ticks require three hosts to complete their life cycle where by each parasitic stage feeds to repletion on distinct host (Walker *et al*., 2003).

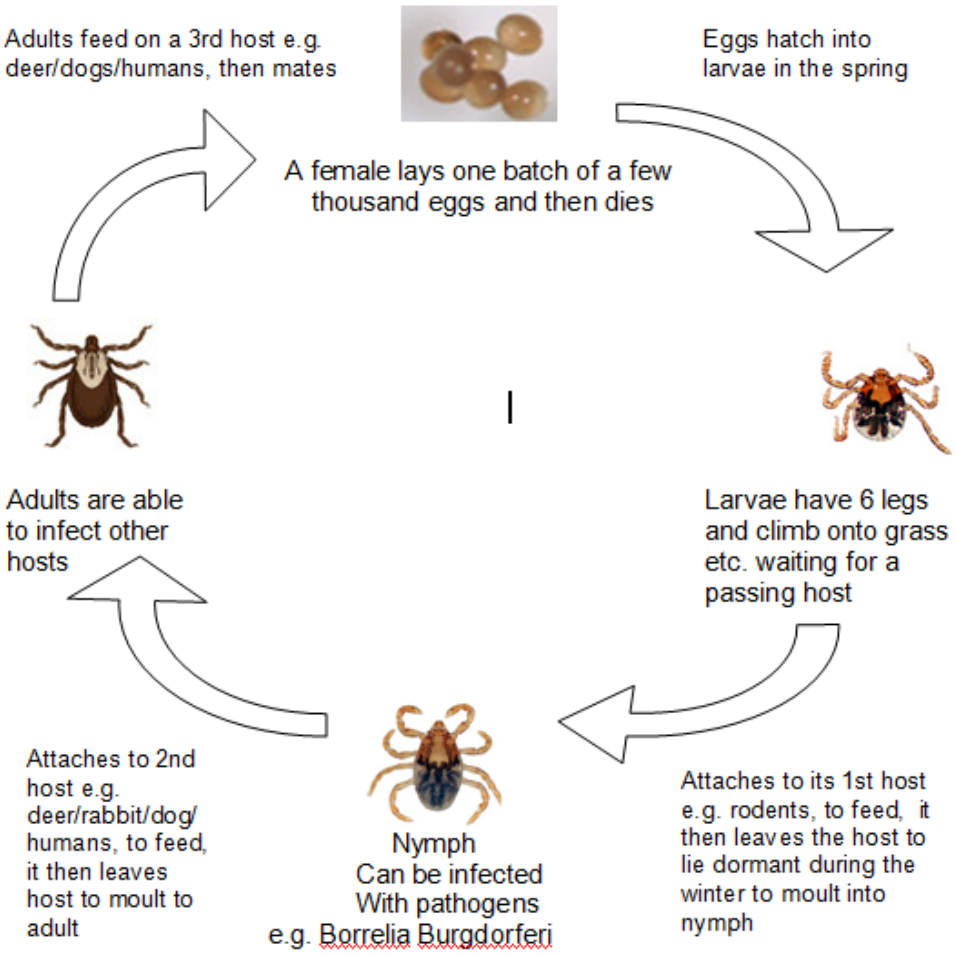


Figure 1: Life cycle of ticks

Tick-borne diseases (TBDs) are reported to be the major limitation to cattle production systems in Africa (Onono *et al*., 2013). Tick-borne diseases of economic important are East Coast fever (ECF), anaplasmosis, babesiosis, and cowdriosis (Kivaria, 2006). The incidence of TBDs and resulting economic losses vary widely within the country due to factors such as agro- ecological zones, cattle type, cattle production systems and socio-economic factors (Kivaria, 2006). East Coast Fever is a cattle disease syndrome caused by the parasite *Theileria parva*, transmitted by *R. appendiculatus* and is one of the most important livestock diseases in Africa (Lessard *et al*., 1988; Norval *et al*., 1991; Muraguri *et al*., 1999). *Theileria parva* depends on the *Rhipicephalus appendiculatus* Neuman 1901, a three-host tick, which parasitizes mainly cattle, for its transmission and its distribution is directly related to the distribution of the ticks. The distribution range of ECF extends from southern Sudan to Africa and as far west as the Democratic Republic of Congo (DRC) (Olwoch *et al*., 2008). Anaplasmosis is a haemoparasitic disease caused by *Anaplasma marginale* a rickettsia that infects the red blood cells of cattle and is transmitted by *Boophilus* spp, cattle ticks common in the warm moist environments (Dalgliesh *et al*., 1990). Anaplasmosis is common tick-borne diseases in Africa, Australia, Russia, South America, and the United States, Mediterranean countries and Russia (Rafyi and Maghami, 1961). Babesiosis is a tick-borne disease of cattle caused by the protozoan parasites of the genus *Babesia* (Ristic, 1981). Parasites of the genus *Babesia* infect a wide variety of domestic and wild mammals as well as man (Penzhorn, 2006). These protozoa particularly *Babesia bovis* and *Babesia bigemina* are transmitted by *Rhipicephalus* spp which are widely distributed in tropical and subtropical countries (Smith *et al*., 1893). *Babesia bovis*, *B. bigemina* together with *B. divergens* are the major inhibitors of the cattle industry and the species are distributed in Africa, Asia, Australia, and Central and South America (Bock *et al*., 2004). Cowdriosis is a tick-borne disease of cattle, sheep, goats and some wild ruminants. The disease is caused by a rickettsia, known as *Ehrlichia ruminantium* which is transmitted by genus *Amblyomma* (*A. variegatum* and *A. hebraeum*) (Provost and Bezuidenhout, 1987). The diseases occur throughout African continent, particularly in South Africa (Okoh *et al*., 1987) and the infection causes a high fever, nervous signs, hydrothorax and oedema of the lungs and brain, and eventually death of the animals.

Tick infestation and tick-borne diseases constrain cattle production and improvement, leading to considerable economic losses. In east Africa, the loss of cattle due to tick and tick borne diseases particularly ECF was estimated to be 168 million United States Dollar including a mortality of 1.1 million cattle (Mukhebi *et al*., 1992). It was also reported that in 1981-1993

tick-borne diseases accounted for 71.4% of annual cattle death in Tanzania. However, in 2006 Kivaria reported that, cattle death due to ECF in Tanzania was estimated to be 68% which attributed to the loss of 364 million USD (Kivaria, 2006). In Uganda the cost incurred for the control of ticks and tick-borne diseases was 308 144 USD (Ocaido *et al*., 2009).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Exudates and ticks collection

Exudates of *C. swynnertonii* were collected from Mererani, Simanjiro district in Arusha region, Tanzania while ticks were obtained from Tanzania Pesticides Research Institute (TPRI). Simanjiro district is located 3°3S' to 36°59E' at an altitude of 980m above the sea level. The plant material was identified in the field by Mr. Innocent Mboya, a botanist from TPRI and voucher specimen coded CS 001 is deposited at the Nelson Mandela African Institution of Science and Technology. Ticks where identified by Mr. Ibrahim an entomologist from TPRI

3.1.1 Extraction of plant materials

Exuates weighed 78.8g were mixed with 150ml of distilled water followed by 150ml of hexane. The solution was thoroughly shaken and allowed to settle in order to form two layers. The hexane layer was separated from aqueous layer by decantation. Thereafter, 150mL of chloroform was poured into the separating funnel containing aqueous layer. The solution were shaken and left for 6h and chloroform layer was separated from the aqueous layer. The two extracts obtained were concentrated through vacuum rotary evaporator, and the extracts obtained were kept in a beaker covered with aluminium foil and stored at 4°C for further use.

3.2 Climbing bioassay

The climbing bioassay was performed as described by Magano and Mkolo (2011). The bioassay is based on climbing behavior of ticks except for the genus *Amblyomma* ticks naturally climb up vegetation to quest for a host (Norval *et al*., 1987). A beaker of 50ml filled with paraffin was firmly inserted in the center of a 250ml beaker which was filled with water to completely surround the small beaker in order to discourage tick from crawling away from paraffin platform (Carrol, 1998). The paraffin provided support to the vertically inserted wood rod (length 11cm) and also served as platform on which ticks were placed. Concentrations of chloroform and hexane extracts were prepared by dissolving 110mg 100mg, 90 mg, 80mg, 70mg, 60mg, 50mg in 10% Dimethyl sulphoxide (DMSO). Two filter papers (12cm^2 each) were prepared on which one filter paper was impregnated with tested extract while the other filter paper was not impregnated with any extract or solvent. The impregnated filter paper was pasted on the top of the wood rod followed by non-impregnated

filter paper which was pasted below it. The same procedures were followed for the positive and negative control. Positive control used was jungle formula commercial insects' repellant and negative control was DMSO. Twenty five *R. appendiculatus* larvae were randomly placed on a paraffin platform of the treated apparatus while the same numbers of larvae were placed on controls platform. The position of the larvae on the wood rod was recorded at 10 minutes intervals for 60 minutes. Ticks (larvae) on the impregnated filter paper and on negative control were considered not repelled while those found on non- impregnated filter paper and on wood rod were considered repelled. Three replications were done for each concentration of the extracts and standard insect repellant (Jungle formula).

3.3 Toxicity of Commiphora swynnertonii extracts using albino mice and rats.

3.3.1 Experimental animals

Albino mice of both sexes, weighted between 19-36 g aged 4 to 5 weeks and albino rats of both sexes weighed between 46-192 aged 7 to 8 weeks were randomly obtained from small animal unit division at the Sokoine University of agriculture (SUA), Morogoro, Tanzania. The animals were allowed to stay in cages with sawdust litters in a controlled temperature of about 25^0C to 29^0C and lightning of 12 h of light and 12 h of darkness for each 24 h period.

3.3.2 Acute oral toxicity

Acute oral toxicity test was done according to Organization for Economic Co-operation and Development (OECD) guidelines number 425 of 2001 (OECD, 2001). The mice were acclimatized for 7 days before the treatments, and they were provided with standard diet and water. The animals were divided into seven groups with six animals each (3 males and 3 females) one, control groups and six treated groups. The mice were fasted 4 h before administration of the extracts. Body weights of the mice were determined and the dose was calculated in accordance with their body weights. One group was given distilled water as controls and the three treated groups were given doses of 500 mg/kg, 1000 mg/kg and 2000 mg/kg body weight of hexane extract while other three groups were given same doses of 500 mg/kg, 1000 mg/kg and 2000 mg/kg body weight of chloroform extract. Food was withheld for about 2 h after administration of the extract but not water. The relevant clinical symptoms were closely observed during the first six hours. The animals were then observed for their toxic symptoms, behavioral changes, and mortality at least once daily for 14 days. After 14 days all mice were weighed and the blood of each mouse was collected through eye orbital

vein into vacutainer tube contained anti-coagulant substance (EDTA). Hematological parameters including white blood cell (WBC), Red blood cell (RBC), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean hemoglobin concentration (MCH), Mean platelet volume (MPV), Mean corpuscular hemoglobin concentration (MCHC), and hemoglobin (Hb).

3.3.3 Sub-acute toxicity

Forty two albino rats were divided into seven groups of six animals each (3 males and 3 females), one control groups and six treated groups. Control group was given distilled water and the three treated groups were given doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight of hexane extract while other three groups were given same doses of 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight of chloroform extract. All administrations were done every 24 h via oral gavage throughout the experimental period. The rats were weighed daily and also subjected to thorough observations for mortality and any behavioral changes, during the 28-day experimental period. After 28 days all rats were weighed and the blood of each rat was collected through eye orbital vein into two vacutainer tubes for each animal. The first vacutainer tube contained anti-coagulant substance (EDTA) and the second vacutainer tube was plain. Hematological parameters including white blood cell (WBC), Red blood cell (RBC), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean hemoglobin concentration (MCH), Mean platelet volume (MPV), Mean corpuscular hemoglobin concentration (MCHC), and hemoglobin (Hb) were determined using the blood samples contained in the EDTA tubes. The blood samples contained in plain vacutainer tubes were centrifuged at 4000 rpm for 10 min and the serum obtained were subjected to biochemical parameters which included total protein, bilirubin, alkaline phosphate (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and albumin. After blood collection, all rats were sacrificed and dissected. The internal organs such as liver, heart, lungs, spleen and kidneys were carefully removed and weighed individually and the relative organ weights were calculated according to the following formula: relative organ weight (%) = weight of organ (g)/body weight (g) × 100. For Histopathological examinations, organs were fixed in 10% formalin before being embedded in paraffin. After routine processing, five micrometers of paraffin sections were prepared and stained with hematoxylin and eosin before microscopic examination.

3.4 Statistical analysis

Results on tick repellency were analyzed using GenStat computer software version 4 (GenStat 4). Effective concentration to repel 50% of the ticks (EC_{50}) was calculated using linear regression equation and percentage repellency was calculated using the formula below as demonstrated by Lima *et al*. (2016).

PR= NTF / (NTF +TF) X 100

Where;

PR is percentage repellent

NTF is number of ticks on non-impregnated filter paper

TF is number of ticks on impregnated filter paper

In acute and subacute toxicity test student's t-test was used to compare initial body weight and final body weight for both control and treated groups of animals while one way analysis of variance (ANOVA) was used in multiple comparisons of the means for hematological, biochemical and organ weight data between the control and treated groups of animals. The statistical analysis was performed using STATISTICA software version 8 (StatSoft, 2007) with the level of significance set at $p< 0.05$.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 Repellency activity of *C. swynnertonii* hexane and chloroform extracts

The repellency activity of *C. swynnertonii* hexane and chloroform extracts were evaluated for repellency activity against *R. appendiculatus* larvae and results obtained are summarized in Tables 1 and 2. The findings from this study indicated that the repellency activity of *C. swynnertonii* hexane and chloroform extracts was concentration and time dependent. The repellency declined as time elapsed (Table 1 and 2). The standard repellant (Jungle formula) used displayed the same activity patterns. It was revealed that there was very high significant difference ($P<0.001$) in repellency effects between control and tested extracts in the first 40 minutes.

Hexane and chloroform extracts had comparable repellency activity as indicated in Table 1. It was evident statistically that there was no significant difference ($P>0.001$) among extracts after 50 and 60 minutes exposure time (Table 1). Furthermore, results revealed that, there was very high significant difference ($P<0.001$) in repellency concentrations of control and tested extracts from 10 to 60 minutes. The concentrations of chloroform and hexane extracts with higher percentage repellency activity comparable to the control were 110 mg/ml and 100 mg/ml (Table 1).

Additionally, findings from this study indicated that there was very high significant interaction effect ($P<0.001$) in both control and treated extracts against concentrations in the first 20 minutes while no significant interaction effect ($P>0.001$) was observed from 30 to 60 minutes.

The concentrations that can repel 50% of the larvae EC_{50} was calculated and results are shown in Table 2. The EC_{50} was generally increasing with an increase in exposure time for both extracts and standard. The EC_{50} could not be calculated at the 10^{th} minutes because all the tested concentrations of the standard repellant had 100% repellency while at the 20^{th} minutes the Y-intercept was greater than 50.

Table 1: Mean percentage repellency of chloroform and hexane C. *swynnertonii* extract against R. *appendiculatus* larvae

Extract	Concentration (mg/ml)	PR (10 min)	PR (20 min)	PR (30min)	PR (40min)	PR (50 min)	PR (60 min)
Chloroform	50	53.63de	44.84hi	24.04ij	10.89j	8.00i	6.72h
	60	58.00bcde	56.06fgh	41.44fghij	32.36ghij	22.27efghi	22.02efgh
	70	61.36bcde	61.94fg	54.04defg	41.58efgh	26.26efghi	23.18efgh
	80	71.45bc	67.13ef	50.40efgh	43.21defh	41.36cdef	27.11efg
	90	73.06b	75.88de	62.36cdeg	55.81cdefg	47.63bcde	36.69def
	100	89.00a	90.60abc	84.07abc	65.36bcde	63.41bcd	54.04bcd
	110	95.41a	92.81ab	87.10ab	74.87bc	71.17ab	57.79bc
Hexane	50	26.34f	21.21j	19.06j	14.65ij	11.06hi	7.32h
	60	47.72e	35.47i	28.26hij	24.51hij	13.71ghi	13.51gh
	70	53.03de	42.93i	39.37ghij	25.23hij	19.93fghi	17.53fgh
	80	55.97cde	46.43hi	41.49fghij	37.48fghi	27.45defgi	20.83efgh
	90	68.61bcd	54.43gh	45.13fghi	40.58fgh	37.96defg	35.04ef
	100	87.85a	77.60de	73.83bce	66.52bcd	65.72abc	56.64bc
	110	94.97a	92.26ab	75.79bc	65.55bcde	70.88ab	62.32b
Control	50	100.00a	76.77de	63.70cdef	25.13hij	23.94defgi	21.89efgh
	60	100.00a	79.66cd	60.49cdeg	42.69defh	30.45defgi	26.39efgh
	70	100.00a	80.42cd	60.65cdeg	56.66cdefg	30.35defgi	32.79efg
	80	100.00a	87.08bcd	76.63bcd	57.24cdef	36.64defh	27.80efg

	90	100.00a	100.00a	89.81a	69.12bc	46.62bcde	29.84efg
	100	100.00a	100.00a	100.00a	88.44ab	49.09bcd	40.54cde
	110	100.00a	100.00a	100.00a	100.00a	88.19a	80.41a
P-value (extracts)		***	***	***	***	ns	ns
P-value (concentration)		***	***	***	***	***	***
P-value (interaction)		***	***	ns	ns	ns	ns

***Significant difference, ns=no significant difference, values followed by different letters denote statistical significance (P<0.001)

Table 2: Effective concentrations (EC_{50}) of chloroform, hexane and control against R. *appendiculatus* larvae at different time intervals

Extracts		Time (Minutes)					
		10	20	30	40	50	60
chloroform	EC_{50}	49.21	55.45	72.44	83.81	89.54	101.22
	R^2	0.9545	0.9785	0.9376	0.9621	0.988	0.9455
Hexane	EC_{50}	68.8	77.37	84.05	91.97	93.71	100.36
	R^2	0.9588	0.9438	0.9206	0.9227	0.9146	0.92
Control	EC_{50}	-	-	42.92	69.13	87.26	97.98
	R^2	-	0.8909	0.8777	0.9704	0.7707	0.6629

R^2 - Regression correlation coefficient; EC_{50} – Concentrations (mg/ml) at which 50% of the *R. appendiculatus* larvae were repelled

4.1.2 Acute toxicity of C. *swynnertonii* hexane and chloroform extract in albino mice

(i) General behavioral changes

The oral administration of *C. swynnertonii* hexane and chloroform extract at 500 mg/kg, 1000 mg/kg and 2000 mg/kg body weight doses had no clinical adverse effect of substance related toxicity and did not cause mortality of any mice during the 14-day observation period.

(ii) Body weight changes

Compared with control there was no significant change ($P>0.05$) in body weight gain or loss of the extract- treated mice throughout the study period (Table 3 and 4). All mice in the group exhibited normal change in body weight without a marked increase or decrease.

Table 3: Body weight (g) values of control and mice treated with C. *swynnertonii* hexane extract measured during the acute toxicity study

Dose (mg/kg BW)	Sex	Mean at day 0	Mean at day 14	P-value
Control	M	28 ±2.52	29.67 ± 0.33	0.547 333
	F	22 ± 2.00	26.33 ± 3.38	0.332 06
500	M	25 ±1.20	26 ±0.58	0.279 44
	F	26 ±1.53	28±0.56	0.287 864
1000	M	28 ±0.67	29 ±0.58	0.534 42
	F	25 ±2.52	26.33±4.37	0.901 236
2000	M	29 ±1.53	29.33 ±2.19	0.155 016
	F	24 ± 2.0	24.67 ± 2.40	0.920 238

Values are expressed as mean ± SEM, M = male, F = female, BW = body weight

Table 4: Body weight (g) values of control and mice treated with C. *swynnertonii* chloroform extract measured during the acute toxicity study

Dose (mg/kg BW)	**Sex**	**Mean at day 0**	**Mean at day 14**	**P-value**
Control	M	28 ±2.52	29.67 ± 0.33	0.547 333
	F	22 ± 2.00	26.33 ± 3.38	0.332 06
500	M	28.33 ± 3.48	31 ± 2.52	0.568 283
	F	28 ± 1.45	30.33 ± 2.33	0.507 158
1000	M	30 ± 1.73	27.67 ± 0.33	0.256 435
	F	29.33 ± 3.53	29 ± 2.31	0.940 784
2000	M	26.67 ± 3.53	22.33 ± 2.73	0.386 239
	F	30.33 ± 2.60	28.67 ± 0.88	0.055 929

Values are expressed as mean ± SEM, M = male, F = female, BW = body weight

(iii) Hematological parameters

The results of hematological parameters of control and treated mice are shown in Table 5 and 6. These results show that there were no significant difference (P>0.05) in all hematological parameters in the group of mice treated with *C. swynnertonii* hexane extract (Table 5) while in the group of mice treated with *C. swynnertonii* chloroform extract showed no significant difference (P>0.05) in hematological parameters except for the mean corpuscular volume (MCV) as indicated in Table 6.

Table 5: Hematological values of control and mice treated with C. *swynnertonii* hexane extract measured during acute toxicity study

Dose (mg/kg BW)						
Parameters	**Sex**	**Control (mg/kg BW)**	**500**	**1000**	**2000**	**P-value**
WBC (m/mm^3)	M	85.57 ± 1.20	85.23 ± 1.22	81.39 ± 3.47	86.62±1.95	0.400 834
	F	85.57 ± 1.20	80.41± 2.48	84.84± 0.84	88.53 ±0.48	0.255 376
RBC $(m/mm)^3$	M	5.75 ± 0.12	8.18 ± 0.01	6.35± 1.44	8.28 ± 0.42	0.101 555
	F	6.50 ± 0.15	3.76 ± 1.00	5.90 ± 1.76	4.59 ± 0.43	0.726 528
MCV (fl)	M	56.37 ± 0.52	56.32 ± 0.59	52.71 ± 2.14	50.73 ± 1.62	0.052 783
	F	52.7 ± 0.06	51 .10± 0.38	53.8 ± 2.47	53.43 ± 0.58	0.107 115
HCT (%)	M	33.43 ± 0.50	44.34 ± 1.19	132.91± 7.61	41.93 ± 2.88	0.191 134
	F	34.23 ± 0.78	19.20± 4.91	30.9 ± 8.50	24.99 ±2.44	0.505 125
MCH (pg)	M	18.75 ± 0.16	18.35 ± 0.09	18.46 ± 0.24	18.33 ± 0.24	0.426 177
	F	22.3 ± 1.45	20.70 ± 1.52	19.7 ± 0.78	19.26 ±0.42	0.145 501
MCHC (g/dl)	M	34.79 ± 0.02	34.83 ± 0.02	34.82 ± 0.02	34.80 ± 0.01	0.496 46
	F	42.77 ± 0.57	45.22± 0.11	45.02 ± 0.29	44.65 ± 0.23	0.220 99
Hb (g/dl)	M	13.00 ± 0.89	14.79 ± 0.20	11.10 ± 2.63	16.03 ± 0.71	0.160 695
	F	14.53 ± 0.99	7.80 ± 1.83	11.4 ± 3.15	9.25 ± 1.05	0.584 332
MPV (fl)	M	8.23 ±0.03	8.46± 0.26	8.30 ± 0.26	8.73 ± 0.03	0.300 601
	F	7.77 ± 0.18	7.50 ± 0.29	8.57 ± 0.43	8.10± 0.06	0.224 104

Values are expressed as mean ± SEM, M= Male, F= Female, BW= Body weight, WBC= white blood cell, RBC=Red blood cell, MCV= Mean corpuscular volume, HCT= Hematocrits, MCH= Mean corpuscular hemoglobin, MCHC= Mean corpuscular hemoglobin concentration, Hb= Hemoglobin, MPV= Mean platelets volume

Table 6: Hematological values of control and mice treated with C. swynnertonii chloroform extract measured during acute toxicity

Dose (mg/kg BW)						
Parameters	**Sex**	**Control**	**500**	**1000**	**2000**	**P-value**
WBC (m/mm^3)	M	85.57 ± 1.20	76.76 ± 22.63	51.64 ± 9.36	84.34 ± 8.52	0.279 208
	F	85.57 ± 1.20	83.81 ± 23.95	52.24 ±1.14	48.69 ± 7.40	0.117 763
RBC $(m/mm)^3$	M	5.75 ± 0.12	4.55 ± 1.24	7.88 ± 0.30	5.57 ± 1.05	0.078 398
	F	6.50 ± 0.15	6.70 ± 0.76	4.53 ± 1.57	6.36 ± 2.60	0.338 938
MCV (fl)	M	56.37 ± 0.52	52 ±4.31	49.73 ± 1.38	50.83 ± 0.66	0.263 628
	F	52.7 ± 0.06^{b}	54 ± 1.75^{ab}	48.25 ± 1.25^{c}	52.5 ± 2.77^{a}	0.016 559
HCT (%)	M	33.43 ± 0.50	23.9 ±6.80	39.2 ± 2.01	26.9 ± 6.27	0.139 489
	F	34.23 ± 0.78	36.37 ± 65.31	22 ± 8.10	33.33 ± 6.14	0.243 186
MCH (pg)	M	18.75 ± 0.16	17.6 ± 1.23	18.1 ± 0.47	18.5 ± 0.19	0.653 304
	F	22.3 ± 1.45	21.3 ± 1.39	18 ± 1.20	21.7 ± 3.11	0.123 996
MCHC (g/dl)	M	34.79 ± 0.02	34.45 ± 3.96	36.37 ± 0.37	36.57 ± 0.22	0.332 826
	F	42.77 ± 0.57	39.83 ± 3.71	37.35 ± 1.45	41.43 ± 3.84	0.123 996
Hb (g/dl)	M	13.00 ± 0.89	8.3 ±2.72	14.27 ± 0.62	10 ± 2.17	0.128 675
	F	14.53 ± 0.99	14.17 ± 1.13	8.35 ± 3.35	13.77 ± 2.97	0.061 809
MPV (fl)	M	8.23 ±0.03	7.97 ±0.47	7.67 ± 0.15	7.4 ± 0.87	0.846 964
	F	7.77 ± 0.18	8.17 ± 0.43	7.8 ± 0.1	7.4 ± 1.83	0.2483

Values are expressed as mean ± SEM, M= Male, F= Female, BW= Body weight, WBC= white blood cell, RBC=Red blood cell, MCV= Mean corpuscular volume, HCT= Hematocrits, MCH= Mean corpuscular hemoglobin, MCHC= Mean corpuscular hemoglobin concentration, Hb= Hemoglobin, MPV= Mean platelets volume, values followed by different letters denote statistical significance ($P<0.001$)

4.1.3 Organ weight

The organ weights relative to body weights of the mice were determined and results are summarized in Table 7 and 8. Findings from this study showed that there were no significant differences (P>0.05) in weight changes of each organ between the control and mice treated with *C. swynnertonii* hexane extract (Table 7) while a significant changes of the vital organs were observed in a group of mice treated with *C. swynnertonii* chloroform extract (Table 8).

Table 7: Organ-body weight values of control and mice treated with C. *swynnertonii* hexane extract measured during acute toxicity study

Dose (mg/kg BW)						
Organ	**Sex**	**Control (mg/kg BW)**	**500**	**1000**	**2000**	**P-value**
Liver	M	4.50 ± 0.04	4.44 ± 0.05	4.34 ± 0.03	4.39 ± 0.03	0.078 648
	F	4.39± 0.02	4.37 ± 0.05	4.41 ± 0.05	4.31 ± 0.02	0.800 511
Spleen	M	0.82 ± 0.002	0.83± 0.005	0.81 ± 0.003	0.81± 0.003	0.061 642
	F	0.82 ± 0.01	0.81± 0.004	0.81 ± 0.003	0.81± 0.003	0.118 330
Heart	M	0.92 ± 0.002	0.91± 0.003	0.91 ± 0.001	0.91± 0.002	0.082 563
	F	0.91 ± 0.001	0.91± 0.003	0.91 ± 0.002	0.91± 0.002	0.835 358
Kidneys	M	0.89 ± 0.01	0.89± 0.004	0.88 ± 0.003	0.87± 0.001	0.241 374
	F	0.89 ± 0.005	0.89 ± 0.01	0.88 ± 0.001	0.88± 0.001	0.090 338

Values are expressed as mean ± SEM, M = male, F = female, BW = body weight

Table 8: Organ-body weight values of control and mice treated with C. *swynnertonii* chloroform extract measured during acute toxicity study

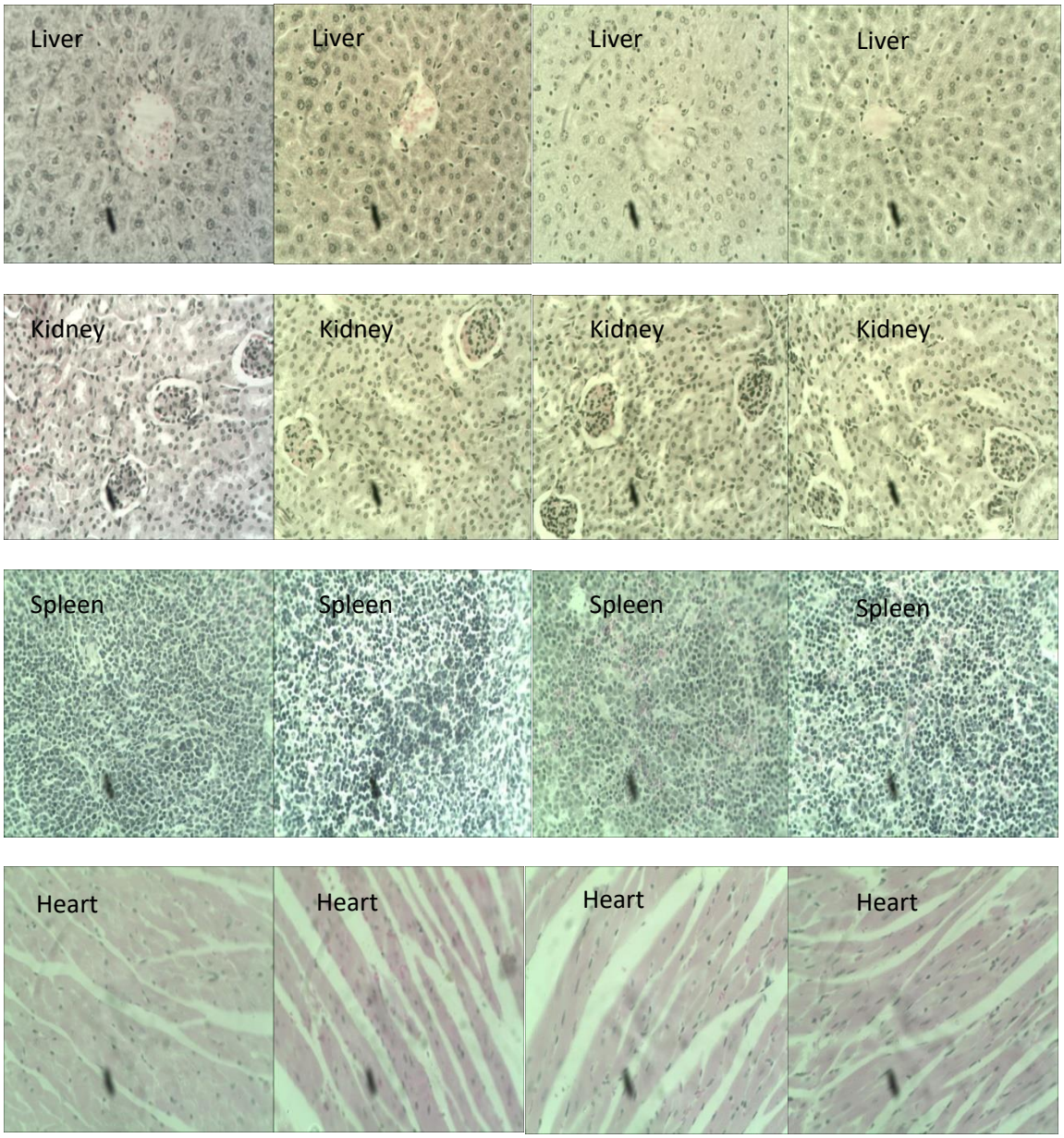
Dose (mg/kg BW)						
Organ	Sex	Control (mg/kg BW)	500	1000	2000	P-value
Liver	M	4.50 ± 0.04^{d}	4.32 ± 0.09^{c}	3.93 ± 0.04^{b}	3.76 ± 0.03^{a}	0.000036
	F	4.39 ± 0.02^{d}	4.09 ± 0.04^{c}	3.91 ± 0.02^{b}	3.65 ± 0.004^{a}	0.000000
Spleen	M	0.82 ± 0.002^{d}	0.81 ± 0.001^{c}	0.81 ± 0.002^{b}	0.79 ± 0.001^{a}	0.000016
	F	0.82 ± 0.01^{c}	0.81 ± 0.001^{a}	0.80 ± 0.0003^{a}	0.79 ± 0.001^{b}	0.000001
Heart	M	0.92 ± 0.002^{a}	0.91 ± 0.001^{a}	0.89 ± 0.003^{a}	0.80 ± 0.04^{b}	0.016468
	F	0.91 ± 0.001^{a}	0.91 ± 0.004^{a}	0.88 ± 0.003^{c}	0.75 ± 0.004^{b}	0.000000
Kidneys	M	0.89 ± 0.01^{b}	0.87 ± 0.01^{ab}	0.85 ± 0.001^{a}	0.82 ± 0.01^{c}	0.000537
	F	0.89 ± 0.005^{a}	0.87 ± 0.004^{a}	0.85 ± 0.001^{a}	0.80 ± 0.002^{b}	0.000000

Values are expressed as mean ± SEM, M = male, F = female, BW = body weight, values followed by different letters denote statistical significance ($P<0.001$)

4.1.4 Histopathology analysis

Results of histopathology analysis of main organs for both control and treated group of mice during acute toxicity are summarized in Fig. 2 and 3. Findings from this study revealed no differences in histopathology examination of the liver, kidney, spleen and heart between control and treated mice with different doses of *C. swynnertonii* hexane extract as shown in Fig. 2. This study further revealed that, the liver and kidney organ of the mice treated with *C. swynnertonii* chloroform extract showed some alteration at different doses while the other organs such as spleen and heart no alteration was observed (Fig. 3). The liver's histopathology analysis of mice treated with a dose of 500 mg/kg *C. swynnertonii* chloroform extract showed portal inflammation with lymphocytes (Fig. 3b). Furthermore, the study revealed minor blood congestion in the portal area of mice treated with 1000 mg/kg *C. swynnertonii* chloroform extract (Fig. 3c). Additionally, a group of mice treated with a dose of 2000 mg/kg *C. swynnertonii* chloroform extract showed vacuolation in the hepatocytes of the liver as shown in Fig. 3d.

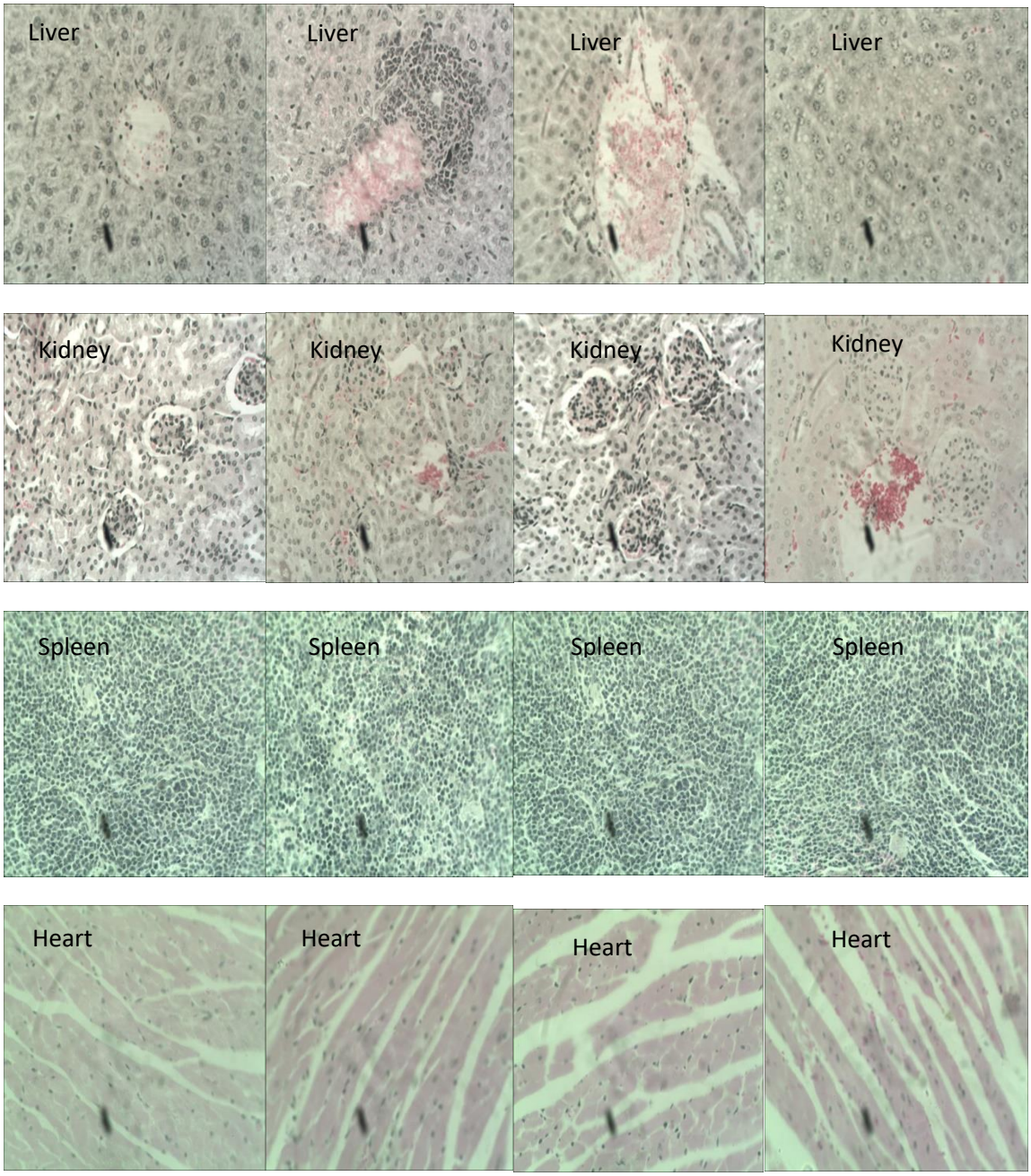
Regarding kidney, animals in a control group showed normal cortex as well as glomeruli and renal tubules exhibited a normal structure (Fig. 3a). Unlike the treated group of mice with dose of 500 mg/kg *C. swynnertonii* chloroform extract, the kidney exhibited a reduction space between glomerular and bowman's capsule as indicated in Fig. 3b. This study also revealed abnormality increase of glomerular in mice treated with dose of 1000 mg/kg *C. swynnertonii* chloroform extract (Fig. 3c) while congestion was shown in group of mice treated with dose of 2000 mg/kg *C. swynnertonii* chloroform extract as shown in Fig. 3d.



(a) (b) (c) (d)

Figure 2: Effects of C. *swynnertonii* hexane extract on histological examinations of the main organs in mice during acute toxicity study.

Representative photomicrographs from liver, kidney, spleen and heart sections stained with hematoxylin and eosin (×40), respective groups: (a) control group (b) treated group (500mg/kg) (c) treated group (1000mg/kg) (d) treated group (2000mg/kg).



(a) (b) (c) (d)

Figure 3: Effects of C. *swynnertonii* chloroform extract on histological examinations of the main organs in mice during acute toxicity study.

Representative photomicrographs from liver, kidney, spleen and heart sections stained with hematoxylin and eosin (×40), respective groups: (a) control group (b) treated group (500mg/kg) (c) treated group (1000mg/kg) (d) treated group (2000mg/kg).

4.1.5 Subacute toxicity of *C. swynnertonii* hexane and chloroform extract in albino rats

(i) General behavioral changes

Daily repeated oral administration of *C. swynnertonii* hexane and chloroform extracts at 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight doses for 28 days did not induce any evident sign of toxicity in the treated animals. No deaths or obvious adverse clinical signs were observed in any of the test groups throughout the experimental period.

(ii) Body weight changes

The mean body weight changes of both control and treated rats are shown in Table 9 and 10. In *C. swynnertonii* hexane extract the significant increase ($P<0.05$) in body weight of male rats was observed in control and at the dose of 250 mg/kg body weight (Table 9) where as in *C. swynnertonii* chloroform extract the significant increase ($P<0.05$) in body weight was observed in control male while at the dose of 500 mg/kg body weight the significant increase ($P<0.05$) in body weight was observed in both male and female rats (Table 10).

Table 9: Body weight (g) values of control and rats treated with C. *swynnertonii* hexane extract measured during sub-acute toxicity study

Dose (mg/kg BW)	Sex	Mean at day 0	Mean at day 28	P-value
Control	M	80 ± 3.00	123 ± 8.00	0.037 287
	F	107 ± 13.00	117.5 ± 20.5	0.707 514
250	M	68 ± 5.00	104.5 ± 5.5	0.039 058
	F	58.5 ±12.5	82 ± 12.00	0.307 850
500	M	87.5 ± 26.5	96.5 ± 30.5	0.844 411
	F	58 ± 2.00	77.5 ± 7.5	0.128 587
1000	M	154.5 ± 37.5	192.5 ± 15.5	0.447 882
	F	115 ± 23.00	121 ± 21.00	0.865 024

Values are expressed as mean ± SEM, M = male, F = female, BW = body weight

Table 10: Body weight (g) values of control and rats treated with C. *swynnertonii* chloroform extract measured during sub-acute toxicity study

Dose (mg/kg BW)	Sex	Mean at day 0	Mean at day 28	P-value
Control	M	80 ± 3.00	123 ± 8	0.037 29
	F	107 ± 13.00	117.5 ± 20.5	0.707 51
250	M	72 ± 7.00	96 ± 20.00	0.374 881
	F	67 ± 8.00	79.5 ± 0.5	0.259 239
500	M	77.5 ± 1.5	104.5 ± 2.5	0.011 460
	F	63 ± 0.00	90.5 ± 3.5	0.015 815
1000	M	116.5 ± 12.5	131 ± 10.00	0.460 646
	F	121.5 ± 21.5	143.5 ± 20.5	0.536 096

Values are expressed as mean ± SEM, M = male, F = female, BW = body weight

(iii) Organ weight

Finding from this study revealed that the weight of the liver treated with *C. swynnertonii* hexane extract was significantly decreased ($P<0.05$) when compared with control while the other organs such as heart, spleen, lung and kidney showed no significant change in weight when compared with control (Table 11). Furthermore, significant decrease ($P<0.05$) in weight of liver, heart, spleen, lung and kidney when compared with control were observed in the rats treated with *C. swynnertonii* chloroform extract (Table 12).

Table 11: Organ-body weight values of control and rats treated with C. *swynnertonii* hexane extract measured during sub-acute toxicity study

Dose (mg/kg BW)						
Organ	**Sex**	**Control**	**250**	**500**	**1000**	**P-value**
Liver	M	3.50 ± 0.11^{a}	2.42 ± 0.28^{b}	3.47 ± 0.60^{a}	3.16 ± 0.28^{a}	0.017 701
	F	4.16 ± 0.48^{a}	4.41 ± 0.48^{a}	3.65 ± 0.01^{a}	1.98 ± 0.22^{b}	0.027 795
Heart	M	0.59 ± 0.04	0.44 ± 0.02	0.63 ± 0.05	0.45 ± 0.03	0.056 310
	F	0.59 ± 0.02	0.58 ± 0.11	0.43 ± 0.06	0.38 ± 0.10	0.298 052
Spleen	M	0.25 ± 0.02	0.21 ± 0.01	0.39 ± 0.05	0.22 ± 0.03	0.058 092
	F	0.32 ±0.02	0.27 ± 0.04	0.26 ± 0.00	0.20 ± 0.05	0.217 631
Lungs	M	0.78 ± 0.18	0.58 ± 0.03	1.18 ± 0.24	0.69 ± 0.14	0.201 823
	F	1.06 ± 0.25	1.09 ± 0.24	0.74 ± 0.12	0.44 ± 0.14	0.120 012
Kidney	M	0.49 ± 0.16	0.32 ± 0.02	0.84 ± 0.16	0.33 ± 0.02	0.092 841
	F	0.48 ± 0.01	0.49 ± 0.11	0.43 ± 0.03	0.29 ± 0.05	0.092 841

Values are expressed as mean ± SEM, M = male, F = female, BW = body weight

Table 12: Organ-body weight values of control and rats treated with C. *swynnertonii* chloroform extract measured during sub-acute toxicity study

Dose (mg/kg BW)						
Organ	**Sex**	**Control**	**250**	**500**	**1000**	**P-value**
Liver	M	3.50 ± 0.11^{b}	2.92 ± 0.21^{a}	2.88 ± 0.05^{a}	2.79 ± 0.04^{a}	0.047 129
	F	4.16 ± 0.48^{b}	3.73 ± 0.28^{a}	3.21 ± 0.15^{a}	3.50 ± 0.17^{a}	0.017 063
Heart	M	0.59 ± 0.04^{b}	0.38 ± 0.00^{a}	0.56 ± 0.01^{b}	0.43 ± 0.02^{a}	0.009 702
	F	0.59 ± 0.02^{b}	0.49 ± 0.07^{ab}	0.30 ± 0.07^{a}	0.40 ± 0.02^{a}	0.044 062
Spleen	M	0.25 ± 0.02^{a}	0.28 ± 0.01a	0.25 ± 0.00^{ab}	0.21 ± 0.02^{b}	0.025 157
	F	0.32 ± 0.02^{b}	0.23 ± 0.01^{a}	0.18 ± 0.01^{a}	0.23 ± 0.02^{a}	0.010 634
Lungs	M	0.78 ± 0.18^{c}	0.54 ± 0.02^{b}	0.79 ± 0.02^{c}	0.63 ± 0.02^{a}	0.005 876
	F	1.06 ± 0.25^{b}	0.95 ± 0.00^{d}	0.85 ± 0.00^{c}	0.74 ± 0.00^{a}	0.000 003
Kidney	M	0.49 ± 0.16^{b}	0.32 ± 0.00^{ab}	0.29 ± 0.01^{a}	0.36 ± 0.01^{c}	0.011 428
	F	0.48 ± 0.01^{a}	0.42 ± 0.01^{ab}	0.49 ± 0.05^{a}	0.36 ± 0.00^{b}	0.042 768

Values are expressed as mean ± SEM, M = male, F = female, BW = body weight, values followed by different letters denote statistical significance ($P<0.001$)

(iv) Hematological parameters

The effects of 28 days administration of *C. swynnertonii* hexane and chloroform extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg body weight doses on the hematological parameters of the animals are represented in Table 13 and 14. With the exception of significant decrease ($P<0.05$) in MCV of female rats, HCT of male rats and significant increase ($P<0.05$) in MPV of female rats treated with *C. swynnertonii* hexane extract, administration of this extract at all the investigated doses had no significant ($P > 0.05$) effect on WBC, RBC, MCH, MCHC and Hb when compared with control (Table 13). Additionally, significant decrease ($P<0.05$) in WBC, RBC, MCV, MCH, Hb and significant increase ($P<0.05$) in MPV was observed in the rats treated with *C. swynnertonii* chloroform extract (Table 14).

Table 13: Hematological values of control and rats treated with C. *swynnertonii* hexane extract measured during sub-acute toxicity study

Dose (mg/kg BW)						
Parameters	**Sex**	**Control**	**250**	**500**	**1000**	**P-value**
WBC (m/mm^3)	M	56.05 ± 0.62	59.91 ± 3.02	52.22 ± 0.24	48.86 ± 0.41	0.063 395
	F	58.43 ± 0.54	48.66 ± 4.71	51.73 ± 1.17	48.33 ± 0.57	0.121 864
RBC (M/mm^3)	M	6.83 ± 0.67	3.61 ± 1.01	14.37 ± 5.05	5.81 ± 0.31	0.082 673
	F	6.33 ± 0.60	5.19 ± 2.05	5.41 ± 0.11	4.36 ± 0.01	0.511 692
MCV (fl)	M	66.15 ± 0.05	58.65 ± 2.65	60.94 ± 0.16	59.6 ± 0.90	0.063 092
	F	61.5 ± 0.40^{b}	59.7 ± 0.80^{a}	60.2 ± 1.00^{a}	61.25 ± 0.36^{a}	0.030 294
HCT (%)	M	37.2 ± 2.70^{a}	20.85 ± 4.95^{b}	35.75 ± 0.15^{a}	34.55 ± 1.35^{a}	0.047 391
	F	38.9 ± 3.90	30.7 ± 11.80	32.5 ± 1.20	26.8 ± 0.10	0.700 330
MCH (pg)	M	23.2 ± 1.90	25.65 ± 3.35	25.24 ± 0.37	24.7 ± 0.70	0.827 095
	F	23.85 ± 0.15	21.8 ± 0.80	26.7 ± 0.70	27.24 ± 0.37	0.061 804
MCHC (g/dl)	M	42.5 ± 2.40	44.15 ± 7.75	41.89 ± 0.21	41.5 ± 0.60	0.966 106
	F	38.9 ± 0.10	36.7 ± 1.80	44.4 ± 0.40	45.06 ± 0.16	0.053 852
Hb (g/dl)	M	15.75 ± 0.25	9.6 ± 3.80	15.35 ± 0.35	14.35 ± 0.35	0.232 666
	F	16.65 ± 0.05	11.5 ± 4.90	14.45 ± 0.65	12.22 ± 0.12	0.632 495
MPV (fl)	M	7.25 ± 0.05	7.3 ± 0.30	14.45 ± 0.05	7.3 ± 0.10	0.389 292
	F	7.5 ± 0.00^{a}	7.3 ± 0.00^{a}	14.45 ± 0.05^{b}	7.5 ± 0.00^{a}	0.021 733

Values are expressed as mean ± SEM, M= Male, F= Female, BW= Body weight, WBC= white blood cell, RBC=Red blood cell, MCV= Mean corpuscular volume, HCT= Hematocrits, MCH= Mean corpuscular hemoglobin, MCHC= Mean corpuscular hemoglobin concentration, Hb= Hemoglobin, MPV= Mean platelets volume, values followed by different letters denote statistical significance ($P<0.001$)

Table 14: Hematological values of control and rats treated with C. *swynnertonii* chloroform extract measured during sub-acute toxicity

	Dose (mg/kg BW)					
Parameters	**Sex**	**Control**	**250**	**500**	**1000**	**P-value**
WBC (m/mm^3)	M	56.05 ± 0.62^{d}	53.06 ± 0.16^{c}	51.22 ± 0.24^{b}	47.61 ± 0.61^{a}	0.000 881
	F	58.43 ± 0.54^{c}	54 ± 0.35^{b}	51.05 ± 1.49^{ab}	47.58 ± 0.82^{a}	0.004 450
RBC (M/mm^3)	M	6.33 ± 0.17^{c}	4.27 ± 1.15^{a}	4.76 ± 0.09^{b}	5.71 ± 0.05^{a}	0.003 540
	F	6.83 ± 0.10^{a}	6.73 ± 0.12^{a}	5.39 ± 0.44^{c}	4.60 ± 0.96^{b}	0.000 032
MCV (fl)	M	66.15 ± 0.05^{d}	62.45 ± 0.35^{b}	63.38 ± 0.18^{c}	60.59 ± 0.01^{a}	0.000 175
	F	61.5 ± 0.40^{a}	58.14 ± 0.27^{a}	60.5 ± 2.20^{a}	60.55 ± 0.05^{b}	0.007 628
HCT (%)	M	37.2 ± 2.70	32.3 ± 1.60	30.85 ± 0.24	34.93 ± 0.13	0.145 418
	F	38.9 ± 3.90	38.80 ± 0.20	26.35 ± 7.95	27.8 ± 5.80	0.310 875
MCH (pg)	M	25.1 ± 0.1^{b}	24.6 ± 0.50^{ab}	23.63 ± 0.07^{a}	24.14 ± 0.06^{a}	0.049 258
	F	23.85 ± 0.15^{ab}	21.93 ± 0.37^{a}	21.9 ± 1.30^{a}	27.3 ± 1.30^{b}	0.042 460
MCHC (g/dl)	M	42.5 ± 2.40	36.35 ± 2.75	37.55 ± 0.05	39.10 ± 0.20	0.239 585
	F	38.9 ± 0.10	38.6 ± 0.30	36.5 ± 3.40	45.2 ± 2.10	0.128 317
Hb (g/dl)	M	15.75 ± 0.25^{c}	12.95 ± 0.65^{b}	11.45 ± 0.05^{a}	10.5 ± 0.20^{a}	0.001 945
	F	16.65 ± 0.05^{d}	14.5 ± 0.30^{c}	13.4 ± 0.30^{b}	9.7 ± 0.20^{a}	0.000 141
MPV (fl)	M	7.25 ± 0.05^{b}	7.65 ± 0.05^{a}	7.65 ± 0.05^{a}	7.55 ± 0.05^{a}	0.013 189
	F	7.5 ± 0.00	7.6 ± 0.00	7.5 ± 0.10	7.35 ± 0.15	0.387 733

Values are expressed as mean ± SEM, M= Male, F= Female, BW= Body weight, WBC= White blood cell, RBC=Red blood cell, MCV= Mean corpuscular volume, HCT= Hematocrits, MCH= Mean corpuscular hemoglobin, MCHC= Mean corpuscular hemoglobin concentration, Hb= Hemoglobin, MPV=Mean platelets volume, values followed by different letters denote statistical significance ($P<0.001$)

(v) Biochemical parameters

Results from serum biochemical parameters of both control and rats treated with *C. swynnertonii* hexane and chloroform extract are described in Table 15 and 16. As compared with control group, significant decreased in AST and creatinine was observed in the female rats treated with *C. swynnertonii* hexane extract (Table 15). Furthermore significant increase ($P<0.05$) in ALT, AST and creatinine was observed in rats treated with *C. swynnertonii* chloroform extract while significant decrease ($P<0.05$) in albumin and significant increase ($P<0.05$) in bilirubin was observed in the male rats treated with *C. swynnertonii* chloroform extract (Table 16).

Table 15: Biochemical parameters of control and rats treated with C. *swynnertonii* hexane extract measured during sub-acute toxicity study

Dose (mg/kg BW)						
Parameters	**Sex**	**Control**	**250**	**500**	**1000**	**P-value**
ALT(u/l)	M	46.66 ± 3.33	45.00 ± 1.67	46.66 ± 0.00	46.66 ± 3.33	0.949 139
	F	54.99 ± 1.67	54.99 ± 8.33	49.99 ± 0.01	69.99 ± 0.00	0.101 273
AST(u/l)	M	88.32 ± 8.33	88.32 ± 31.66	119.99 ± 0.00	63.33 ± 10.00	0.280 390
	F	84.99 ± 1.67^{d}	76. 66 ± 3.33^{c}	53.33 ± 3.33^{a}	66.67 ± 0.01^{b}	0.002 884
ALP(u/l)	M	262.58 ± 135.44	253.686 ± 3.37	250.41 ± 0.09	210.188 ± 0.12	0.944 925
	F	323.39 ± 66.34	187.95 ± 96.74	100.89 ± 17.97	69.99 ± 0.00	0.121 238
Albumin(g/dl)	M	3.94 ± 0.05	3.00 ± 0.78	3.40 ± 0.00	3.04 ± 0.03	0.407 136
	F	3.71 ± 0.02	4.15 ± 0.60	3.79 ± 0.65	2.61 ± 0.01	0.223 086
Bilirubin(mol/l)	M	1.24 ± 0.32	1.08 ± 0.12	0.97 ± 0.01	1.07 ± 0.49	0.931 227
	F	1.24 ± 0.60	0.79 ± 0.77	0.66 ± 0.08	0.62 ± 0.00	0.798 284
T. protein(g/dl)	M	7.16 ± 0.11	7.61 ± 0.76	8.74 ± 0.00	7.00 ± 0.64	0.200 357
	F	7.14 ± 0.49	6.52 ± 0.44	6.12 ± 0.53	6.91 ± 0.01	0.438 118
Creatinine(u/l)	M	0.67 ± 0.08	0.67 ± 0.08	0.58 ± 0.00	0.62 ± 0.04	0.744 777
	F	0.54 ± 0.04^{a}	0.49± 0.05^{a}	0.54 ± 0.04^{a}	0.33 ± 0.00^{b}	0.049 440

Values are expressed as mean ± SEM, M = Male, F = Female, BW = Body weight, ALP = Alkaline phosphate AST =Aspartate aminotransferase, ALT= Alanine aminotransferase, values followed by different letters denote statistical significance (P<0.001)

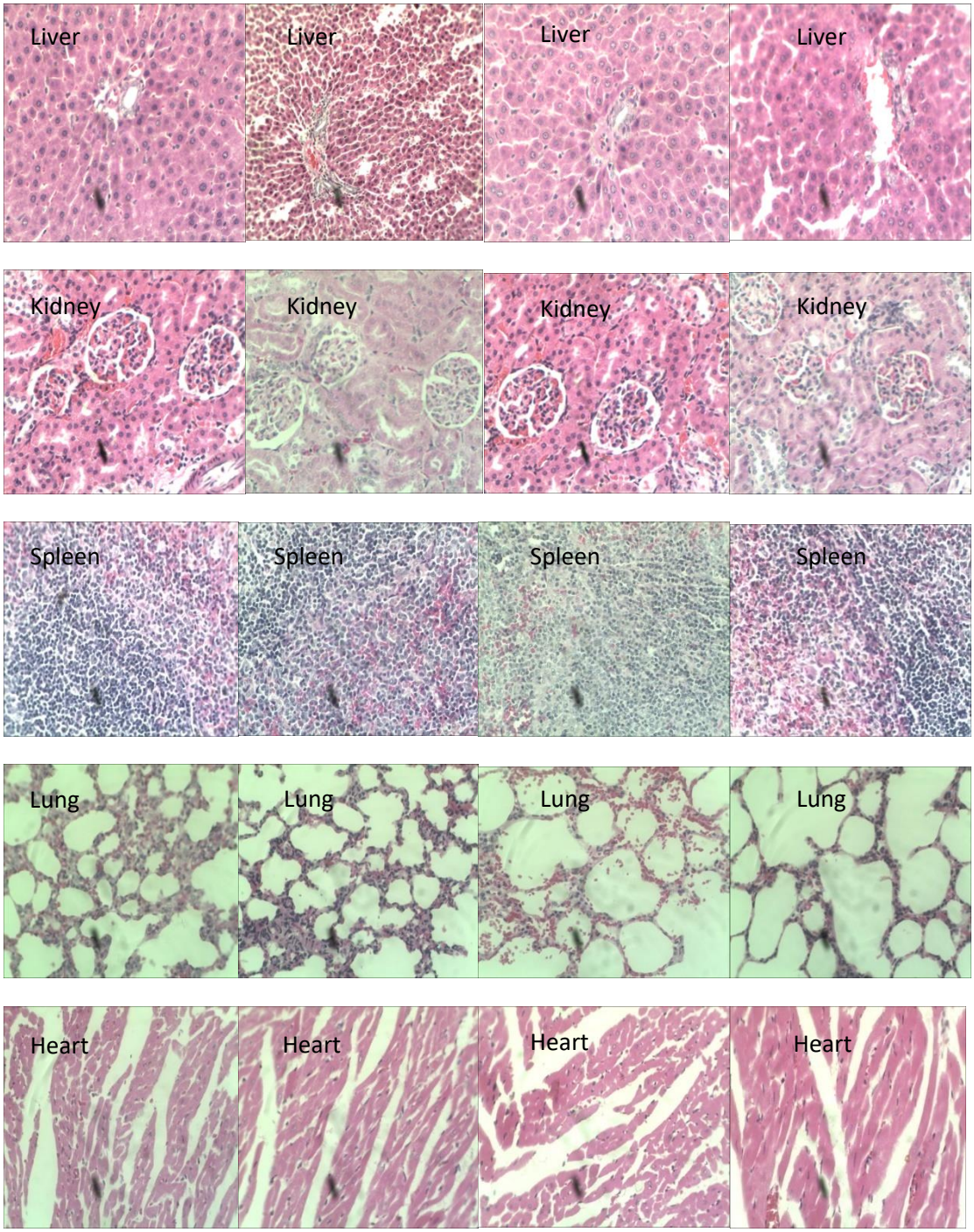
Table 16: Biochemical parameters of control and rats treated with C. *swynnertonii* chloroform extract measured during sub-acute toxicity study

Dose (mg/kg BW)						
Parameters	**Sex**	**Control**	**250**	**500**	**1000**	**P-value**
ALT(u/l)	M	46.66 ± 3.33[a]	54.99 ± 8.33[a]	82.82 ± 0.51[b]	59.99 ± 0.01[a]	0.018 493
	F	54.99 ± 1.67[a]	43.33 ± 0.00[b]	58.33 ± 1.67[a]	68.33 ±1.67[c]	0.000 994
AST(u/l)	M	88.32 ± 8.33	81.66 ±1.67	89.44 ± 0.56	90.17 ± 0.51	0.540 147
	F	84.99 ± 1.67[a]	149.99 ± 0.00[c]	96.66 ± 3.34[b]	87.60 ± 0.50[a]	0.000 047
ALP(u/l)	M	262.58 ± 135.44	382.81 ± 134.05	391.99 ± 0.50	425.66 ± 0.00	0.669 992
	F	323.39 ± 66.34[b]	304.04 ± 0.00[b]	152.02 ± 11.06[a]	149.26 ± 2.76[a]	0.037 294
Albumin(g/dl)	M	3.94 ± 0.05[a]	3.81 ± 0.25[a]	3.55 ± 0.00[a]	4.70 ± 0.01[b]	0.011 852
	F	3.71 ± 0.02	3.29 ± 0.00	3.85 ± 0.30	3.95 ± 0.01	0.121 126
Bilirubin(mol/l)	M	1.24 ± 0.32[b]	2.53 ± 0.07[a]	3.09 ± 0.15[a]	3.57 ± 0.42[a]	0.015 113
	F	1.24 ± 0.60	1.26 ± 0.50	2.10 ± 0.28	2.59 ± 0.10	0.187 983
T. protein(g/dl)	M	7.16 ± 0.11	8.51 ± 0.95	7.19 ± 0.01	7.83 ± 0.01	0.294 342
	F	7.14 ± 0.49	7.67 ± 0.00	7.68 ± 0.10	8.12 ± 0.22	0.240 198
Creatinine(u/l)	M	0.67 ± 0.08	0.79 ± 0.04	0.82 ± 0.00	0.94 ± 0.02	0.294 615
	F	0.54 ± 0.04[b]	0.83 ± 0.00[a]	0.85 ± 0.00[a]	0.92 ± 0.03[a]	0.002 078

Values are expressed as mean ± SEM, M = Male, F = Female, BW = Body weight, ALP = Alkaline phosphate AST =Aspartate aminotransferase, ALT= Alanine aminotransferase, values followed by different letters denote statistical significance ($P<0.001$)

(vi) Histopathological analysis

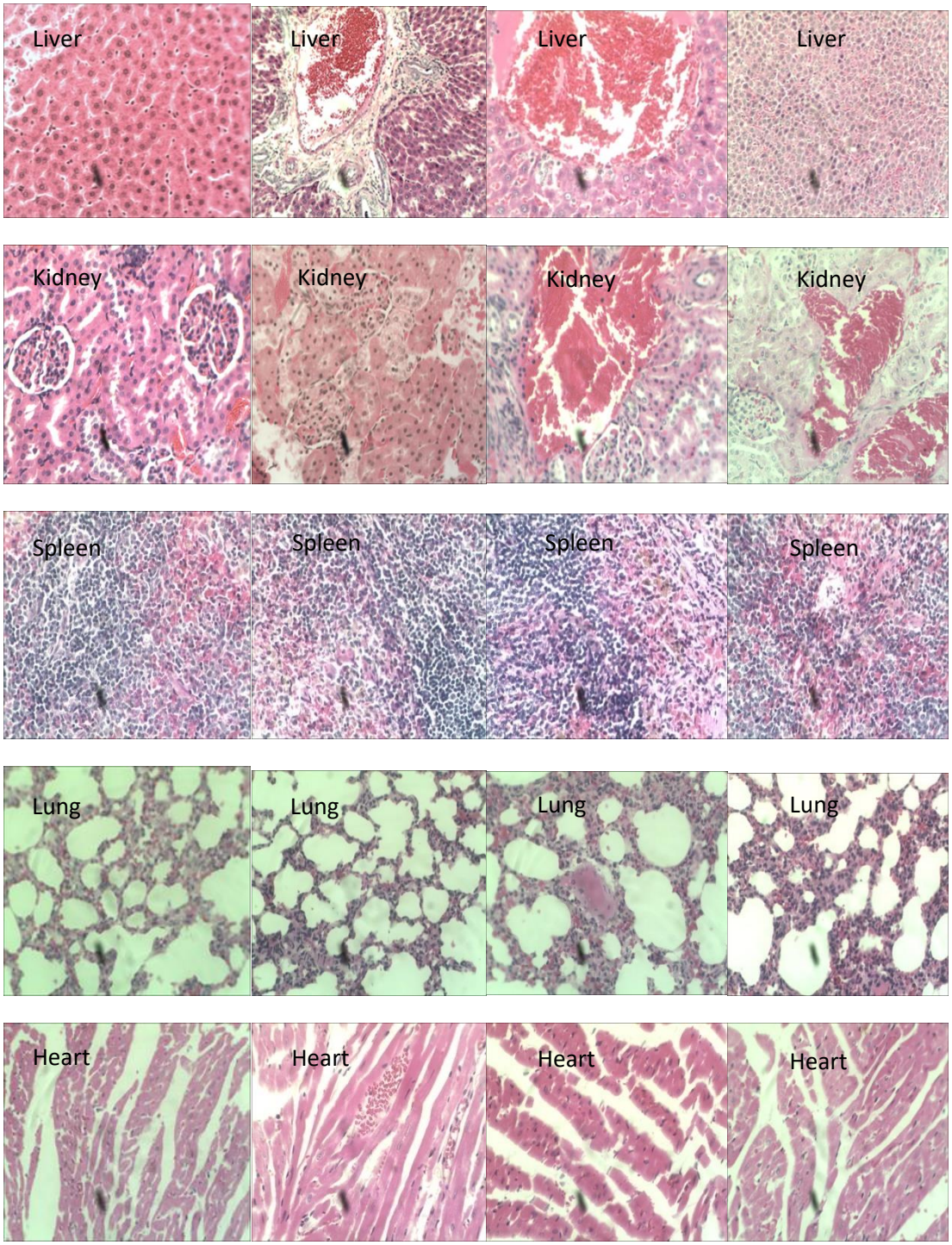
Microscopic examinations of the liver, kidney, spleen, lung and heart of the animals treated with *C. swynnertonii* hexane extract revealed no abnormalities in overall structural orientation of the organs during the 28 days of repeated dose toxicity study when compared with normal control (Fig. 4). The histopathological examination of the liver treated with *C. swynnertonii* hexane extract showed normal hepatic architecture, hepatocytes and perilobular vein. However, the histological analysis of the kidney of the animals treated with *C. swynnertonii* hexane extract revealed normal renal architecture with a normal appearance of glomerulus. The spleen sections displayed normal architecture with lymphoid follicles and sinuses. Assessment of the lung presented normal appearance with normal bronchioles and alveolus. Also the histology examination of the heart displayed normal myocardial architecture. Furthermore there were some changes in the structures of some organ's sections of the animals treated with *C. swynnertonii* chloroform extract as comparable to the normal control (Fig. 5). The examination of liver section of the animals treated with *C. swynnertonii* chloroform extract revealed moderate to severe congestion and initial stage of necrosis (Fig. 5). Also the kidney section of the animals treated with *C. swynnertonii* chloroform extract induced congestion and destruction in the glomerulus's shape (Fig. 5). The other organs such as spleen, lung and heart displayed normal structures.



(a) (b) (c) (d)

Figure 4: Effects of C. *swynnertonii* hexane extract on histological examinations of the main organs in the rats during subacute toxicity study.

Representative photomicrographs from liver, kidney, spleen, lung and heart sections stained with hematoxylin and eosin (×40), respective groups: (a) control group (b) treated group (250mg/kg) (c) treated group (500mg/kg) (d) treated group (1000mg/kg).



(a) (b) (c) (d)

Figure 5: Effects of C. *swynnertonii* chloroform extract on histological examinations of the main organs in the rats during subacute toxicity study.

Representative photomicrographs from liver, kidney, spleen, lung and heart sections stained with hematoxylin and eosin (×40), respective groups: (a) control group (b) treated group (250mg/kg) (c) treated group (500mg/kg) (d) treated group (1000mg/kg.

4.2 Discussion

Medicinal plants have been used worldwide for thousands of years for treatment of both human and animal ailments (Gurib, 2006; Bakari, 2013). The treatment ability of these plants is due to presence of different secondary metabolites produced by different morphological parts of the plants. Genus *Commiphora* produces secondary metabolites which proved to be invaluable in drugs development (Zorloni, 2008). The presence of secondary metabolites from *C. swynnertonii* was found to be responsible for antimicrobial and anti-parasitic activities (Bakari *et al*., 2012). This study evaluated the repellency and toxicity of *C. swynnertonii* hexane and chloroform extracts.

4.2.1 Repellency activity of *C. swynnertonii* hexane and chloroform extracts

Repellency is caused by the release of volatile secondary metabolites which are known to cause disorders in the movement of the target species away from the odor source (Jaenson *et al*., 2005). Plants with insecticidal repellency effects were the main insecticidal agents in Africa for management of insect vectors before the introduction of synthetic insecticidal agents. Validation of such plants has been viewed as the best option of developing bio-pesticides that are readily available in developing countries. The *Commiphora swynnertonii* exudate is widely used by pastoralists for the management of ticks. It has therefore ethnomedical and ethnoveterinary significance in Tanzania which necessitated scientific validation of the exudates. Results emanated from this study demonstrated that hexane and chloroform extracts of *C. swynnertonii* exudates has repellency activity against *R. appendiculatus* larvae. Both *C. swynnertonii* chloroform and hexane extracts exhibited repellency activity in all concentrations (50 mg/ml, 60 mg/ml, 70 mg/ml, 80 mg/ml, 90 mg/ml, 100 mg/ml and 110 mg/ml) which indicated that the activity was caused by release of volatile compounds that cause the targeted organism to move against the odor source. This study confirms that the *Commiphora* species contain volatile substances that are responsible for the repellency effect that can be extracted with different organic solvents. Findings from this study were comparable to the results obtained from standard insects' repellant (Jungle formula). The observation was supported by Lwande *et al*. (1999) findings who reported that the essential oil of *Gynandropsis gynadra* was compared to the commercially synthetic arthropod repellant in repelling *Rhipicephalus appendiculatus* ticks. These findings clearly suggest that some plant species may contain anti-tick agents that may be equally effective against ticks as some of the commercially synthetic arthropod repellants. The effectiveness of

the extract against *R. appendiculatus* larvae was dose dependent as evidenced by an increase in percentage repellency as the concentration increased from 50 mg/ml to 110 mg/ml.
The lower concentrations appeared to be less effective since the amount of volatile compound which enhances the repellency activity was lower than in higher concentration. However, the effectiveness of the extracts against *R. appendiculatus* was decreased as time declined from 10 to 60 minutes. EC_{50} appeared to be increasing as the time increases for both tested extracts and the standard insects' repellant. The lowest EC_{50} appeared to be the best concentration in repelling the ticks since it induces high repellency effect within a short period of time and also minimize the utilization of plant products and hence, conservation of *C. swynnertonii* diversity.

Findings from the current study on the repellency properties exhibited by *C. swynnertonii* exudates contribute to the body of knowledge on the insecticidal properties of the genus *Commiphora*. Furthermore, findings from this study validate the ethnoveterinary information on the use of *C. swynnertonii* exudate on the management of tick infestation on livestock among Maasai community in Tanzania.

4.2.2 Acute toxicity of *C. swynnertonii* hexane and chloroform extract in albino mice

Toxicity using animal models is very essential in order to assess potential risks of the chemical compounds in the plant which could result in adverse effects in human health (Afolayan *et al*., 2016; Bello *et al*., 2016; Schulz *et al*., 2001). In this study, oral administration of *C. swynnertonii* hexane and chloroform in albino mice at 500 mg/kg, 1000 mg/kg and 2000 mg/kg for 14 days had no effects on mortality, examined clinical signs, or any behavioral changes. No acute toxicity was found in mice treated with *C. swynnertonii* extracts, so the approximate lethal dose was determined to be higher than 2000 mg/kg. *C. swynnertonii* hexane and chloroform extracts showed normal change in body weight without a marked increase or decrease. Since no significant changes in body weight were observed in both control and treated animals, this study therefore suggests that the tested extracts had no effects on the body weights of the mice. Blood is an important index of physiological status in both man and animals and the parameters measured usually are white blood cell (WBC), Lymphocyte(LYM), Monocytes (MON), Neutrophil (NEU), Red blood cell (RBC), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean hemoglobin concentration (MCH), Red blood cell distribution width (RDW), Mean platelet volume (MPV), Mean corpuscular hemoglobin concentration (MCHC), and hemoglobin (Hb) (Vaghasiya *et al*.,

2011). Alteration of these parameters can be caused by ingestion of some toxic plants (Ajagbonna *et al*., 1999). This study has shown that acute oral ingestion of *C. swynnertonii* hexane extract did not induce significant change in all hematological parameters for both control and treated mice. However, mean corpuscular volume (MCV) had shown slightly changes in group of mice treated with *C. swynnertonii* chloroform extract. The reason for this slightly changes might be due to inhibition in blood cells synthesis by the active constituents of *C. swynnertonii* chloroform extract.

Organ weight is an important index to diagnose whether the organ was exposed to the injury or not (Jothy *et al*., 2011). The primary organs which are mostly affected by toxic substances are liver, heart, kidney, lungs and spleen (Naidu *et al.*, 2014; Kilonzo *et al*., 2016). In this study, the weights of internal organs were not statistically significant in both control group and treated group of mice treated with C. *swynnertonii* hexane extract indicating that the extract is virtually nontoxic. The non-toxicity shown by *C. swynnertonii* hexane extract towards albino mice, ratify the safety profile of the hexane extract of the plant. However, this study observed significant decrease in liver, heart, spleen and kidney weight of mice treated with *C. swynnertonii* chloroform extract indicating that chloroform extract contain some toxic substances that affected the weight of internal organs of albino mice.

Histopathological analysis provides information on morphological changes in tissue sections by light microscopy (Naidu *et al*., 2014). Microscopic examination of internal organs of the animals treated with *C. swynnertonii* hexane extract in this study did not reveal any abnormalities for both control and treated group and therefore suggest that *C. swynnertonii* hexane extract is potentially safe for human consumption. On the other hand, administration of *C. swynnertonii* chloroform extract at different doses resulted in abnormalities such as portal inflammation with lymphocytes, minor blood congestion in the portal area and vacuolation in the hepatocytes of livers in the treated mice. These abnormalities in the liver might be caused by nonpolar compounds present in the *C. swynnertonii* extract. Histological examination of kidney section of mice treated with *C. swynnertonii* chloroform extract at all dosses revealed reduction space between glomerular and bowman's capsule, abnormality in glomerulus's shape and congestion. These changes confirm that the kidney tissue was damaged by chloroform extract. These results collaborate with the previous study conducted by Mwanaisha *et al*. (2015) on the antimicrobial and cytotoxicity efficacy of *C. swynnertonii* extracts which showed that chloroform extract was toxic in sea brine shrimps.

4.2.3 Subacute toxicity of C. swynnertonii hexane and chloroform extract in albino rats

Since treatment-related toxicity was not evidenced during the acute toxicity evaluation, further testing was conducted to evaluate the 28-day repeated daily dose of the extracts on key metabolic markers of the animals. The sub-acute toxicity study, which involved rats given *C. swynnertonii* hexane and chloroform extract orally at doses of 250, 500 and 1000 mg/kg body weight generally, elicited no clinical signs of toxicity, mortality or any behavioral changes. Additionally the results from this study indicated that *C. swynnertonii* hexane and chloroform extract induced normal change in body weight and therefore it can be suggested that the tested extracts had no effects on the body weights of the rats.

Regarding the organ weight during the 28 days of oral administration of *C. swynnertonii* extracts, findings from this study revealed that, with the exception of significant decreased in the weight of the liver, the weights of other internal organs such as heart, spleen, lung and kidney were not statistically significantly in both control group and treated groups of rats treated with *C. swynnertonii* hexane extract. The decrease in the weight of liver in the rats treated with *C. swynnertonii* hexane extract was not corroborated by both the histological examination and other clinical biochemical parameters of liver function and therefore indicating that the extract is virtually nontoxic. However this study observed significant decrease in liver, heart, spleen, lung and kidney weight of rats treated with *C. swynnertonii* chloroform extract indicating that chloroform extract contain some toxic substances that affected the weight of internal organs of the rats.

Blood parameters such as white blood cell (WBC), Red blood cell (RBC), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean hemoglobin concentration (MCH), Red blood cell distribution width (RDW), Mean platelet volume (MPV), Mean corpuscular hemoglobin concentration (MCHC), and hemoglobin (Hb) can be used to determine the extent of the deleterious effect of foreign compounds in plant extracts in the blood (Ashafa *et al*., 2009). In this study, the nonsignificant difference in WBC, RBC, MCH, MCHC, and Hb following repeated daily dose treatment with *C. swynnertonii* hexane extract could be an indication that the hexane extract it may not be toxic to the blood. However regarding the rats treated with *C. swynnertonii* chloroform extract, some of the parameters including WBC, RBC, MCV, MCH, Hb and MPV were significantly different from those of the control group. Reductions in RBC, MCV, MCH and Hb indicated that the extract interfered with the normal production of hemoglobin and its concentrations within red blood cells. Thus, it can be suggested that *C.*

swynnertonii chloroform extract may possess the potential to induce anemia (Amna *et al*., 2013).

Moreover, the observed reductions in WBC in the rats treated with *C. swynnertonii* chloroform extract may suggest a decline in the function of the immune system. Therefore, these results suggest that the *C. swynnertonii* chloroform extract has a tendency to cause anemia and immunological defects in rats, rendering the animals more vulnerable to infections. Evaluation of serum biochemical parameters and examining major toxic effects on specific tissues may provide useful information regarding the overall health status and alteration in metabolic processes of the animals caused by ingestion of plant extracts (Yamthe *et al*., 2012; Li *et al*., 2017). The liver and the kidneys are target organs for toxic chemicals due to their essential functions in biological detoxification and excretion processes (Bello *et al*., 2016). When the liver cell membrane is damaged, a variety of enzymes normally located in the cytosol are released into the blood stream. The transaminases (AST and ALT) and PLA are well-known enzymes used as good indicators of liver function (Ismail *et al*., 2014). ALT is plenty in the cytoplasm of liver cells and has been commonly used as a marker to quantify suspected liver cell damage (Giannini, 2005; Ferreira, 2014).

Since AST found in the serum is of both mitochondrial and cytoplasmic origin and any rise can be considered as a first sign of cell damage that leads to the outflow of the enzymes into the serum (Mukinda and Eagle, 2010). ALP is often employed to assess the integrity of the plasma membrane of the liver (Onu *et al*., 2013).The kidneys excrete metabolic waste products and regulate the serum concentration of a variety of substances (Benouadah *et al*., 2016). Creatinine as well as urea is important biomarkers of renal toxicity (Santosh and Pravin, 2016) and increase in the levels of these parameters indicates a marked renal damage (Suryavanshi *et al*., 2015). Results from this study showed that oral administration of *C. swynnertonii* hexane extract to albino rats revealed the insignificant changes of most of the serum biochemical parameters which suggest the nontoxic nature of the extract. Though there were some minor changes in AST and creatinine for female, these parameters were significantly decreased when compared with control group.

Therefore it is still suggest the nontoxic nature of hexane extract. Furthermore significant increases in the level of some biochemical parameters particularly ALT, AST and creatinine for female, albumin and bilirubin were observed in the rats treated with *C. swynnertonii* chloroform extract as compared with control. Significant decrease in ALP level for female

was also observed in the rats treated with *C. swynnertonii* chloroform extract. The increase in the level of ALT, AST as well as bilirubin in the rats treated with *C. swynnertonii* chloroform extract may indicate liver damage probably by altered cell membrane permeability leading to the leakage of the enzymes from the tissues to the serum.

Histopathological result of liver sections of rats treated with *C. swynnertonii* chloroform extract confirmed these effects indicating normal to severe congestion and initial stage of necrosis. However, regarding the kidney function, the significant increase in serum level of creatinine in the test groups treated with *C. swynnertonii* chloroform extract suggest possible renal damage. Histological aspects indicated a severe congestion and destruction of glomerular shape in the kidney section treated with *C. swynnertonii* chloroform extract.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The use of plant-based products, particularly those from indigenous plants, may minimize the costs incurred by countries to import synthetic acaricides. The results from this study strengthen the view that *C. swynnertonii* is the potential source of anti-tick agents and this validates the ethno veterinary use of the plant for management of tick infestation on the animals. The results of this study, further strengthens the widely held view that plant products can be used as an alternative to synthetic tick-repellents.

The study evaluated the effect of single dose and daily repeated doses of *C. swynnertonii* hexane and chloroform extracts using albino mice and rats. The results indicated that LD_{50} of *C. swynnertonii* hexane and chloroform extracts is above 2000 mg/kg body weight. Following its 28-day repeated daily oral dose administration of *C. swynnertonii* hexane and chloroform extracts in the animals, it may be concluded that *C. swynnertonii* hexane extract does not elicit any treatment-related adverse effect at the investigated doses and thus may be classified to be relatively safe and virtually nontoxic for consumption. However, the observed changes in the hematological, serum biochemical parameters which signifies liver and kidney damage lead to alterations in the normal physiological functions of vital organs and weakening of the immune system of the animals, if there is prolonged consumption of *C. swynnertonii* chloroform extract. Therefore, caution and safety measures should be taken for oral ingestion of *C. swynnertonii* chloroform extract for therapeutic purposes or for other uses; and prolonged use should be discouraged. The use of the plant for management of ticks is of no consequences if only the users are assured that the extracts are not ingested.

5.2 Recommendation

This study intended to contribute towards the knowledge base of plant species with therapeutic potential. Five recommendations are therefore advanced as follows:

i) Further on-station and field studies based on repellency and acaricidal activity of *C. swynnertonii* chloroform and hexane extracts are needed
ii) Dissemination of the findings regarding the potential of *C. swynnertonii* extracts to the pastoralists should be practiced.
iii) Further studies are highly suggested to screen *C. swynnertonii* from different parts of

the country to identify the diversity of secondary metabolites and test for efficacy and safety of pure isolated secondary metabolites of the plants in the management of ticks

iv) Conservation of *C. swynnertonii* should be an important aspect to ensure sustainable availability of the plant. Therefore, agronomical studies should be carried out on propagation and on whether it can be introduced in other geographical areas

v) Since *C. swynnertonii* chloroform extract showed some toxic properties in the tested animals, the extract can be isolated, identified and evaluated for other uses

REFERENCE

Adam, M. E. and Selim, S. A. (2013). Antimicrobial activity of essential oil and methanol extract from *Commiphora molmol* (Engl.) resin." *International Journal of Current Microbiology and Applied Sciences.* **2**(2): 1-6.

Adebayo, A. H., Aliyu, R., Gatsing, D. and Garba, I. H. (2006). The Effects of Ethanolic Leaf Extract of *Commiphora africana* (Burseraceae) on Lipid Profile in Rats. *International Journal of Pharmacology.* **2:** 618-622.

Afolayan, A. J., Wintola, O. A. and Fouche, G. (2016). Acute and Subacute Toxicological Evaluation of the Aerial Extract of *Monsonia angustifolia* E . Mey . ex . A . Rich in Wistar Rats. *Evidence-Based Complementary and Alternative Medicine,* **2016:** 1-8.

Ajagbonna, O. P., Onifade K. I. and Suleiman, U. (1999). Hematological and biochemical changes in rats given extract of *Calotropis procera. Sokoto Journal of Veterinary Sciences.* **1**(1)**:** 36-42.

Akor, J. S. and Anjorin, T. S. (2009). Phytochemical and antimicrobial studies of *Commiphora africana* root extracts. *International Journal Agriculture and Biology.* **11:** 795–797.

Al-Harbi, M.M., Qureshi, S., Raza, M, Ahmed, M. M., Afzal, M. and Shah, A. H. (1997). Gastric antiulcer and cytoprotective effect of *Commiphora molmol* in rats. *Journal of Ethnopharmacology.* **55:** 141-150.

Al-Howiriny, T., Al Sohaibani, M., Al Said, M., Al Yahya, M., El Tahir, K. and Rafatullah, S. (2005). Effect of *Commiphora opobalsamum* (L.) Engl. (Balessan) on experimental gastric ulcers and secretion in rats. *Journal of Ethnopharmacology.* **98** (3): 287-294.

Aliyu, R., Adebayo A. H., Gatsing, D. and Garba, I. H. (2007). The effects of leaf extract pharmacology of *Commiphora africana* (Burseraceae) on rat liver and kidney function. *Journal of Pharmacology and Toxicology.* **2:** 373-379.

All, A. S. and AIi, M. A. Z. (2007). Antibacterial Activity of *Commiphora quadricincta* from Saudi Arabia. *Journal of King Saud University.* **1** (12): 1420-2000.

Al-Mathal, E. M. and Fouad, M. A. (2004). Myrrh (*Commiphora molmol*) in treatment of human and sheep *Dicrocoeliasis dendriticum* in Saudi Arabia. *Journal of Egypt Social Parasitology*. **34** (2): 713-20.

Amna, O. F., Nooraain, H., Noriham, A., Azizah A. and Husna, R. N. (2013). Acute and oral subacute toxicity study of ethanolic extract of Cosmos caudatus leaf in Sprague Dawley rats. *International Journal of Bioscience, Biochemistry and Bioinformatics.* **3**(4): 301–305.

Ashafa, A. O., Yakubu, M. T., Grierson, D. S. and Afolayan, A. J. (2009). Effects of aqueous extract from the leaves of *Chrysocoma ciliata* L. on some biochemical parameters of Wistar rats. *African Journal of Biotechnology*. **8**(8): 1425–1430.

Bakari, G. G, Max, R. A, Mdegela, H. R, Phiri, E. C. and Mtambo, M. M. (2012). Effect of crude extracts from *Commiphora swynnertonii* (Burtt) against selected microbes of animal health importance. *Journal of Medicinal Plants Research.* **6**(9): 1795-1799.

Bakari, G. G. (2013) In: Kaoneka, B., Mollel M. and Lyatuu F. (2007). Leaf essential oil *composition and tick repellency activity of Commiphora swynnertonii Burtt. Journal of Biological Research.* **8:** 213–216.

Bakari, G., Max, R., Mdegela, R., Phiri, E. and Mtambo, M. (2011). Antimicrobial and Antifungal activity of Commiphora swynnertonii (Burt) against selected pathogens of public health importance. *Research Journal of Biological Sciences*. **6**(4): 175-179

Baki, M. A., Khan, A., Al-Bari, M. A., Mosaddik, A, Sadik, G. and Mondal, K. A. (2007). Sub-acute toxicological studies of Pongamol isolated from *Pongamia pinnata*. *Research Journal of Medical Sciences*. **2:** 53-57.

Bekalo, I. M., Keengwe, E., Mathias, P. M. and Mundy, P. (1996). Ethnoveterinary medicine in Kenya. A field manual of traditional animal health care practice. *Intermediate Technology Development Group and International Institute of Rural Reconstruction*. pp. 226.

Belguet, A. (2010). Evaluation of toxicity in mice and rats and antioxidant activities of *Ruta montana* leaves extracts. PhD thesis, University of Ferhat Abbas Sétif 1, Algeria, pp.18-37.

Bellamkonda, R., Karuna, R., Sreenivasa, R. S., Ramesh, B. K., Ramatholisamma, P., Appa, R. C. and Saralakumari, D. (2011). Anti-hyperglycemic and antioxidant activities of alcoholic extract of *Commiphora mukul* gum resin in streptozotocin induced diabetic rats. *Pathophysiology.* **18** (4): 255-261.

Bello, I., Bakkouri, A. S., Tabana, Y. M., Al-Hindi, B., Al-Mansoub, M. A., Mahmud, R. and Asmawi, M. Z. (2016). Acute and sub-acute toxicity evaluation of the methanolic extract of alstonia scholaris stem bark. *Medical Sciences*. **4**(1): 1-4.

Benouadah, Z., Mahdeb N. and Bouzidi, A. (2016). Evaluation of Acute and Sub-Acute Toxicity of Alkaloids from *Datura stramonium* Sp. in Mice. *International Journal of Pharmacognosy and Phytochemical Research.* **8**(11): 1759–1766.

Birkett, M. A., Al Bassi, S., Kröber T., Chamberlain K., Hooper A. M., Guerin P. M., Pettersson J., Pickett J. A., Slade R. and Wadhams L. J. (2008). Anti ectoparasitic activity of the gum resin, gum haggar, from the East African plant, *Commiphora holtziana*. *Phytochemistry.* **69**(8): 1710-1715.

Bock, R., Jackson, L., de Vos, A. and Jorgensen, W. (2004). Babesiosis of cattle. *Parasitology.* **129:** 247-269.

Carballo, J. L., Hernández-Inda, Z. L., Pérez P. and García-Grávalos, M. D. (2002). A comparison between two brines shrimp assays to detect in vitro cytotoxicity in marine natural products. *BMC Biotechnology.* **2:** 1-17.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Review.* **12:** 564-582.

Dalgliesh, R. J., Jorgensen, W. and De Vos, A. J. (1990). Australian frozen vaccines for the control of babesiosis and anaplasmosis in cattle—A review. *Tropical Animal Health and Production*. **22**(1): 44–52.

Dharmananda, S. (2003). Myrrh and frankincense, spiritual significance. *Internet Journal*. **2:** 1- 6.

Duffus, J., Nordberg, M. and Templeton, D. (2009). Glossary of terms used in toxicology, 2nd edition (IUPAC recommendation 2007). *Pure Applied Chemistry*. **79**(7): 1153-1344.

Ezekiel, I., Mabrouk, M. A. and Ayo, J. O. (2010). Study of the Effect of Hydro- Ethanolic Extract of Commiphora africana (Stem-bark) on Inflammation and Pain in Rodents. *Asian Journal of Medical Sciences.* **2**(3): 81-84.

Fatope, M. O., Al-Burtomani, S. K. S., Ochei, J. O., Abdulnour, A. O., Al-Kindy, S. M .Z. and Takeda, Y. (2003). Muscanone: a 3-O-(1″, 8″, 14″-trimethylhexadecanyl) naringenin from *Commiphora wightii*. *Phytochemistry.* **62**(8): 1251-1255.

Ferreira, S. A., Guimarães, A. G., Ferrari, F. C., Carneiro, C. M., Paiva N. C. N. D. and Guimarães, D. A. S. (2014). Assessment of acute toxicity of the ethanolic extract of *Lychnophora pinaster* (Brazilian arnica). *Revista Brasileira de Farmacognosia.* **24:** 553–560.

George, J. E., Pound, J. M. and Davey, R. B. (2004). Chemical control of ticks on cattle and the resistance of these parasites to acaricides. *Parasitology*. **129:** 353–366.

Giannini, E. G., Testa R. and Savarino, V. (2005). Liver enzyme alteration, a guide for clinicians. *Canadian Medical Association Journal.* **172:** 367–379.

Goji, A. D. T., Dikko, A. A. U., Bakari, A. G., Mohammed, A. and Tanko, Y. (2009). Evaluation of the Effect of Aqueous-ethanolic Stem Bark Extract of Commiphora africana on Blood Glucose Levels of Alloxan Induced Diabetic Wistar Rats. *Asian Journal of Medical Sciences.* **1**(2): 18-21.

Goldman, P. (2001). Herbal medicines today and the roots of modern pharmacology. *Annals of Internal Medicines*. **135**(8): 594-600.

Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. *Molecular Aspects of Medicine*. **27**(1): 1-93.

Haffor, A. S. (2010). Effect of myrrh (*Commiphora molmol*) on leukocyte levels before and during healing from gastric ulcer or skin injury. *Journal of Immunotoxicology.* **7**(1): 68-75

Hanus, L. O., Rezanka, T., Dembitsky, V. M. and Moussaieff, A. (2005). Myrrh — *Commiphora*, chemistry. *Biomedical Paper.* **149:** 3–28.

Haridy, F. M., Dawoud, H. A. and Morsy, T. A. (2004). Efficacy of *Commiphora molmol* (Mirazid) against sheep naturally infected with *Monieziasis expansa* in Al-Santa Center, Gharbia Governorate, and Egypt. *Journal of Egypt Social Parasitology*. **34**(3): 775-82.

Higa, L. D. O. S., Garcia, M. V., Barros, J. C., Koller, W. W. and Andreotti, R. (2015). Medicinal chemistry Acaricide Resistance Status of the *Rhipicephalus microplus* in Brazil : A Literature Overview. *Medicinal Chemistry. **5*:** 326–333.

Horak, I. G., Fourie, L. J., Heyne, H., Walker, J. B. and Needham, G. R. (2002). Ixodid ticks feeding on humans in South Africa: with notes on preferred hosts, geographic distribution, seasonal occurrence and transmission of pathogens. *Experimental and Applied Acarology*. **27:** 113-136.

Iqbal, R. Z., Song-hua, H. U., Wan-jun, C. and Chen-wen, X. (2006). Importance of ticks and their chemical and immunological control in livestock. *Journal of Zhejiang University SCIENCE B.* **7**(11): 912–921.

Ismail, Z., Halim, S. Z., Abdullah, N. R., Afzan, A., Abdul Rashid B. A. and Jantan, I. (2014). Safety Evaluation of Oral Toxicity of *Carica papaya* Linn. leaves: A Subchronic Toxicity Study in Sprague Dawley Rats. *Evidence-Based Complementary and Alternative Medicine.* **2014:** 1-10.

Jongejan, F. and Uilenberg, G. (2004). The global importance of ticks. *Parasitology*. **129:** 3-14.

Jothy, L. S., Zakaria Chen, Z., Ling Lau, Y., Yoga Latha, L. and Sasidharan S. (2011). Acute oral toxicity of methanolic seed extract of *Cassia fistula* in mice. *Molecule*. **16**(6): 5268-5282.

Kalala, W. M., Magadula, J. J. and Mdegela, R. H. (2014). Ethnobotanical use of *Commiphora swynertonii* Burrt . amongst Dorobo people in Tanzania. *Journal of Medicinal Plant Research*. ***8***(23): 820–828.

Kalala, W., Magadula J. and Mdegela, H. (2014). Evaluating Acaricidal Activity of *Commiphora swynertonii* (Burrt) bark Exudate against common Ticks in Tanzania. *International Journal of Herbal Medicine*. **2**(4): 19–25.

Kaoneka, B., Mollel M. and Lyatuu F. (2007). Leaf essential oil composition and tick repellency activity of *Commiphora swynnertonii* Burtt. *Journal of Biological Research.* **8:** 213–216.

Kivaria, F. M. (2006). Estimated direct economic costs associated with tick-borne diseases on cattle in Tanzania. *Tropical Animal Health and Production*. ***38***(4): 291–299.

Kivaria, F. M., Noordhuizen, J. P. T. M. and Msami, H. M. (2007). Risk factors associated with the incidence rate of clinical mastitis in smallholder dairy cows in the Dar es Salaam region of Tanzania. *Veterinary Journal*. **173**(3): 623–629.

Kumari, R., Meyyappan, A., Nand, D., Bikram K. A., Avik, A. C., Palanisamy, S., Subbaiah, L., Venkatachalam, S. G., Joydeep, M., Santu, B. and Parasuraman J. (2011). Antioxidant and antibacterial activities of bark extracts from Commiphora berryi and *Commiphora caudata*. *Natural Product Research*. ***25***(15): 1454-1462.

Latif, A. A. and Walker, A. R. (2004). An introduction to the biology and control of ticks in Africa, ICTTD-2 project. 1-29.

Lessard, P., Sorensen, M., Norval, R. A. I., Perry, B. D., Dolan, T. T., Burrill, A. L., Eplattenier, R., Grootenhuis, J. G. and Irvin, A. D. (1988). Geographical information systems in the estimation of East Coast fever risk to African livestock. *Acta Veterinaria Scandinavica.* **84:** 234–236.

Li, F., Li, Y., Li, Q., Shi X. and Guo, Y. (2017). Acute and Subacute Oral Toxicity Evaluation of *Eriobotrya japonica* Leaf Triterpene Acids in ICR Mice. *Evidence-Based Complementary and Alternative Medicine.* **2017:** 1-9.

Massoud, A. M., El-Shazly, A. M., Awad, S. E., Morsy, A. T., Sadek, G. S. and Morsy, T. A. (2006). New trends in diagnosis and treatment of chronic intestinal strongyloidiasis stercoralis in Egyptian patients. *Journal of Egypt Society of Parasitology*. **36:** 827–44

Mekonnen, Y., Aman, D. and Dagne, E. (2003). toxicity study in mice of resins of three. *Ethiopan Journal of Science*. **26**(2): 151–153.

Meyer, B. N., Ferrigni, N. R., Putnam, J. E., Jacobsen, L. B., Nichols, D. J. and McLaughlin, J. L. (1982). Brine shrimp: a convenient general bioassay for active plant constituents. *Planta Medica*. **45**(5): 31-34.

Minja M. M. J. (1999). The Maasai wander plants. Paper presented at the people and plants training workshop held at the tropical pesticide research institute Arusha, Tanzania 15th to 18th March, 1999.

Mkangara, M., Erasto P. and Chacha, M. (2014). Acaricidal Activity of *Commiphora Swynnertonii* (Burtt) Stem Bark Extracts against adult *Rhipicephalus Appendiculatus* Newman and *Amblyomma Variegatum. American Journal of Research Communication.* **2**(9): 82–92.

Mkangara, M., Erasto P. and Chacha, M. (2014). Antimicrobial and Cytotoxicity Efficacy of *Commiphora swynnertonii* (Burtt) Extracts. *International Journal of Science and Research.* **3:** 2319-7064.

Mkolo, M. N. and Magano, S. R. (2007). Repellent effects of the essential oil of Lavendula angustifolia against adults of Hyalomma marginatum rufipes. *Journal of the South African Veterinary Association*. ***78***(3): 149-152.

Moreira, D. D., Teixeira, S. S., Monteiro, M. H., DeOliveira, A. C. and Paumgartten, F. J. (2014). Traditional use and safety of herbal medicines. *Revista Brasileira de Farmacognosia*. **24**(2): 248-257.

Mukhebi, A.W., Perry, B. D. and Kruska, R. (1992). Estimated economics of theileriosis control in Africa. *Preventive Veterinary Medicine*. **12**(1): 73–85.

Mukinda J. T. and Eagles, P. F. (2010). Acute and sub-chronic oral toxicity profiles of the aqueous extract of *Polygala fruticosa* in female mice and rats. *Journal of Ethnopharmacology.* **128:** 236-40.

Muraguri, G. R., Kiara, H. K. and McHardy, N. (1999). Treatment of East Coast fever: a comparison of parvaquone and buparvaquone. *Veterinary Parasitology.* **87:** 25–37

Musa, U., Abdu, P. A., Dafwang, I. I., Katsayal, U. A., Edache, J. A. and Karsin, P. (2008). Ethnoveterinary Remedies Used for The Management of Newcastle Disease in Some Selected Local Government Areas of Plateau State Nigeria. *Nigerian Journal of Pharmaceutical Sciences.* **7**(1): 126– 130.

Naidu, J., Ismail, R. and Sasidharan, S. (2014). Acute Oral Toxicity and Brine Shrimp Lethality of Methanol Extract of Mentha Spicata L (Lamiaceae). *Tropical Journal of Pharmaceutical Research*. **13:** 101–107.

Nasri, H. and Shirzad, H. (2013). Toxicity and safety of medicinal plants. *Journal of Herbal Medicines and Plarmacology*. **2**(2): 21-22.

Newton, S. M., Lau, C., Gurcha, S. S., Besra, G. S. and Wright, C. W. (2002). The evaluation of forty-three plant species for in vitro antimycobacterial activities; isolation of active constituents from Psoralea corylifolia and Sanguinaria canadensis. *Journal of Ethnopharmacology*. **79**(1): 57-67

Norval, R. A. I., Perry, B. D. and Young, A. S., (1992). The Epidemiology of Theileriosis in Africa. Academic Press, London.

Nyabayo, C. T., Matasyoh, J. C. and Mwendia, C. (2015). Chemical composition and acaricidal activity of Salvia nilotica essential oil against *Rhipicephalus appendiculatus*. **3:** 46–54.

Ocaido, M., Muwazi, R. T. and Opuda, J. A. (2009). Economic impact of ticks and tick-borne diseases on cattle production systems around Lake Mburo National Park in South Western Uganda. *Tropical Animal Health and Production*. **41**(5): 731–739.

Okoh, A. A. E. J., Oyetunde, I. L. and Ibu, J. O. (1987). Heartwater infection (cowdriosis) in a sitatunga (tragelaphus spekei) in Nigeria. *Journal of Wildlife Diseases*. **23**(2): 211–214.

Olwoch, J. M., Reyers, B., Engelbrecht, F. A. and Erasmus, B. F. N. (2008). Climate change and the tick-borne disease , Theileriosis (East Coast fever) in sub-Saharan Africa. *Journal of Arid Environments*. **72:** 108–120.

Onono, J. O., Wieland, B. and Rushton, J. (2013). Constraints to cattle production in a semiarid pastoral system in Kenya. *Tropical Animal Health and Production*. **45:** 1415–1422.

Onu, Y., Saidu, M. J., Ladan, L. S., Bilbis, A. A., Aliero S. M. and Sahabi. (2013). Effect of Aqueous Stem Bark Extract of *Khaya senegalensis* on Some Biochemical, Haematological, and Histopathological Parameters of Rats. *Journal of Toxicology.* **2013:** 1-9.

Opdebeeck, J. P., Wong, J. Y., Jackson, L. A. and Dobson, C. (1988). Hereford cattle immunized and protected against Boophilus microplus with soluble and membrane-associated antigens from the midgut of ticks. *Parasite Immunology*. **10**(4): 405–410.

Paraskeva, M. P., Van Vuuren, R. L. Van Zyl, H. D. and Viljoen, A. M. (2008). In vitro biological activity of selected South African *Commiphora* species. *Journal of Ethnopharmacology.* **119:** 673–679.

Penzhorn, B. L. (2006) Babesiosis of wild carnivores and ungulates. *Veterinary parasitology*. **138**(2): 11-21.

Provost, A. and Bezuidenhout, J. D. (1987). The historical background and global importance of heartwater. *Journal of Veterinary Research*. **54:** 165–169.

Rafyi, A. and Maghami, G. (1961). Contribution a l'etude de quelques parasites du sang du mouton et de la chevre en Iran et dans les pays voisins. *Bulletin Office International Epizootise.* **65:** 1769–1783

Rao, R. M., Khan, Z. A. and Shah, A. H. (2001). Toxicity studies in mice of *Commiphora molmol* oleo – gum – resin, *International Journal of Pharmaceutical Sciences Review and Research.* **76:** 151–154.

Ristic, M. and McIntyre, I. (1981). Infectious Diseases of Cattle in the Tropics, 1st Edition (Eds.); Martinus Nijhoff Publishers: The Hague. **6:** 443-468.

Ruffo, C. K., Birnie A. A. and Tengnas, B. (2002). Edible wild plants of Tanzania," *Regional Land Management Unit/Sida*, Nairobi Kenya, pp. 766

Sambuta A. K. and Masola, S. N. (2006). The efficacy of *Commiphora swynnertonii* extracts in the control of external parasites in livestock. *Proceedings Papers of COSTECH,* pp. 42-43

Santosh N. B. and Pravin, D. C. (2016). Acute and Sub-Acute Otal Toxicity Assessment of the Polyherbal Formulation in Albino Wister Rats. *International Journal of Pharmacy and Pharmaceutical Sciences.* **8:** 311-316.

Silva Lima, D., Carva Lho, J. F. D., Peixoto, M., Blank, A., Borges, L. M. and Costa Junior, L. (2016). Assessment of the repellent effect of Lippia alba essential oil and major monoterpenes on the cattle tick Rhipicephalus microplus. *Medica and Veterinary Entemology*. **30:** 73–77.

Sindhu, Z., Jonsson, N. N. and Iqbal, Z. (2003). Discovery of New Ixodicides : from Documentation of Ethno-veterinary Practices to Scientific Validation in Laboratory, pp. 1–19.

Singh, R. B., Niaz, M.A. and Ghosh, S. (1994). Hypolipidemic *Commiphora mukul* therapy in patients and antioxidant effects of as an adjunct to dietary with hypercholesterolemia. *Cardiovascular Drugs and Therapy*. **8:** 659-664.

Soromessa, T. (2013). Perspective Ecological Phytogeography : A Case Study of *Commiphora* Species, *Science, Technology and Arts Research Journal*. ***2***(3): 93-104.

Suleiman, M. H. A (2014). Prenylated flavonoids from the stem wood of *Commiphora Opobalsamum* (L) Engl. (Burseraceae). *Journal of King Saud University –Sciences*. **27**(1): 71-75

Suryavanshi, S. A., Shinde-Kadam, K., Raina, P., Nimbargi, R., Pandit V. A. and Kaul-Ghankar, R. (2015). Evaluation of Acute and Sub-Acute Toxicity of a Standardized Polyherbal Formulation (HC9): An In Vivo Study. *International Journal of Pharmacy and Pharmaceutical Sciences.* **7:** 110-117.

Urizar, N. L., Liverman, A. B., Dodds, D. T., Silva, F. V., Ordenlich, P., Yan, I., Gonzalez, F. J., Heyman, R. A., Mangelsdorf, D. J. and Moore, D. D. (2002). A natural product that lowers cholestrol antagonistic ligand for FXR. *Journal of Science.* **296:** 1703 - 1706.

Vaghasiya, Y., Shukla V. and Chanda, S. (2011). Acute Oral Toxicity Study of Pluchea arguta Boiss Extract in mice. *Journal of Pharmacology and Toxicology.* **6**(2): 113-123

Van Der Walt, J. J. A. (1974). A preliminary report on the genus *Commiphora* in South West Africa. *Madoqua*. **1**(8): 5-23.

Walker, A. R., Bouattour, J. L., Camicas, A., Estrada – pena, A., Horak, I.G., Latif, A.A., Pegram, R.G. and Preton, P. M. (2003). *Ticks of domestic animals in Africa: A guide to identification of species*, Bioscience Report, The University of Edinburgh, Scotland, UK, pp. 3-210.

Wanzala, W., Hassanali, A., Mukabana, W. R. and Takken, W. (2014). Repellent Activities of Essential Oils of Some Plants Used Traditionally to Control the Brown Ear Tick, *Rhipicephalus appendiculatus*. *Journal of Parasitology Research*. **2014:** 1-10.

Yamthe, L. R., David K. and Ngadena, Y. M. (2012). Acute and Chronic Toxicity Studies of the aqueous and ethanol leaf extracts of *Carica papaya* Linn in Wistar rats. *Journal of Natural Products*. **2:** 617–627.

Zhu, N., Rafi, M. M., DiPaola, R. S., Xin, J., Chin, C. K., Badmaev, V., Ghai, G., Rosen, R.T. and Ho, C.T. (2001). Bio-active constituents from gum guggul (*Commiphora wightii*). *Phytochemistry*. **56:** 723-727.

Zorloni, A. (2008). Evaluation of plants used for the control of animal ectoparasitosis in southern Ethiopia (Oromiya and Somali regions). Magister Scientiae thesis, University of Pretoria, South Africa, pp.13-40.

RESEARCH OUTPUT

Published Papers

1. Repellency activity of *Commiphora swynnertonii* exudates against *Rhipicephalus appendiculatus* larvae.

2. Acute and Subacute Toxicity of *Commiphora swynnertonii* extracts: An Experimental Study on Albino Mice and Rats.

3. Contribution of natural products in the management of livestock diseases.

INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 11, No. 1, p. 281-286, 2017
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Repellency activity of *Commiphora swynnertonii* exudates against *Rhipicephalus appendiculatus* larvae

Disela Edwin[1], Paul Erasto[2], Sylvester Temba[1], Musa Chacha[*1]

[1]*School of Life Sciences and Bio-Engineering, Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania*

[2]*National Institute of Medical Research, Dar es salaam, Tanzania*

Article published on July 30, 2017

Key words: *Commiphora swynnertonii,* Repellency activity, Climbing bioassay

Abstract

Evaluating plant extracts for anti-tick properties is essential towards development of alternative method for controlling tick infestation and other harmful insects. This study aimed to evaluate repellency activity of *Commiphora swynnertonii* hexane and chloroform extracts against *Rhipicephullus appendiculatus* larvae using climbing bioassay method. Concentrations of 110mg/ml, 100 mg/ml, 90mg/ml, 80mg/ml, 70mg/ml, 60mg/ml and 50mg/ml of hexane and chloroform extracts were used. *C. swynnertonii* chloroform and hexane extract had repellency activity in all concentrations at all-time intervals. The repellency activity was appeared to be concentration and time dependent. The results from this study strengthen the view that *C. swynnertonii* is the potential source of anti-tick agents.

***Corresponding Author:** Musa Chacha ✉ musa.chacha@nm-aist.ac.tz

Introduction

The use of synthetic acaricides is the most common method used for decades by livestock keepers to control both hard and soft ticks (Opdebeeck *et al*., 1988). Synthetic acaricides that has been employed for the management of ticks are organophosphates (OP), phyrethroid (SP), amitraz, macrocyclic lactone (ML), Arsenic, organochlorine, and benzenehexachloride (BHC), polychloroterpine, dieldrin and aldrin, cyclodiene compounds and toxaphene just to mention a few. Despite the fact that synthetic acaricides has been effective in the management of ticks, their use has become less reliable, acceptable and sustainable due to several reasons, such as high cost of acaricides, tick resistance to synthetic acaricides and contamination of the environment or food with toxic residues (Mkolo *et al*., 2007; Bissinger *et al*., 2010; Kalala *et al*., 2014;). These challenges promote the necessity to seek for alternative tick control methods which is affordable, effective, ecofriendly and less toxic to the livestock and livestock keepers.

The use of natural substances such as plant-based products to kill or repel parasitic arthropods on livestock have been widely used by many communities as an alternative to synthetic acaricides (Opiro *et al*., 2013). However scientific studies have been conducted by scientific researchers to validate the ethno veterinary use of plant based products which are used by indigenous people in the communities. For example, a study conducted by Silva Lima *et al*., (2016) on repellency activity of *Lippie alba* essential oils growing in Brazil found to possess repellency activity against *Rhipicephalus appendiculatus*. On another study, Wanzala *et al*., (2014) observed repellency activity of *Tagetes minuta* and *Tithonia diversifolia* growing in Kenya against *R. appendiculatus*.

Commiphora swynnertonii (Burseraceae) is a small highly branched and thorny tree with a height of about 3 meters tall. The plant is widely distributed in Africa and Asia and is among the plant species commonly used in Tanzania and Kenya for the management of ticks (Kalala *et al*., 2014).

Although several studies have been carried out to assess and validate the medicinal value of *Commiphora* species (Aliyu *et al*., 2002), only little information on *C. swynnertonii* is available in the literature. This paper therefore reports the repellency activity of *C. swynnertonii* extracts against *R. appendiculatus* larvae.

Materials and methods

Exudates and ticks collection

Exudates of *C. swynnertonii* were collected from Mererani, Simanjiro district in Arusha region, Tanzania while ticks were obtained from Tanzania Pesticides Research Institute (TPRI). The plant material was identified in the field by Mr. Innocent Mboya, a botanist from TPRI and voucher specimen coded CS 001 is deposited at the Nelson Mandela African Institution of Science and Technology.

Extraction of plant materials

Exudates weighed 78.8g were mixed with 150ml of distilled water followed by 150ml of hexane. The solution was thoroughly shaken and allowed to settle in order to form two layers. The hexane layer was separated from aqueous layer by decantation.

Thereafter, 150ml of chloroform was poured into the separating funnel containing aqueous layer. The solution were shaken and left for 6 hours and chloroform layer was separated from the aqueous layer. The two extracts obtained were concentrated through vacuum rotary evaporator, and the extracts obtained were kept in a beaker covered with aluminium foil and stored at 4°C for further use.

Climbing bioassay

The climbing bioassay was performed as described by Magano and Mkolo, (2011). The bioassay is based on climbing behavior of ticks. Except for the genus *Amblyomma* ticks naturally climb up vegetation to quest for a host (Norval *et al*., 1987).

A beaker of 50ml filled with paraffin was firmly inserted in the center of a 250ml beaker which was filled with water to completely surround the small beaker in order to discourage tick from crawling away from paraffin platform (Carrol, 1998).

The paraffin provided support to the vertically inserted wood rod (length 11cm) and also served as platform on which ticks were placed. Concentrations of chloroform and hexane extracts were prepared by dissolving 110mg 100mg, 90mg, 80mg, 70mg, 60mg, 50mg in 10% Dimethyl sulphoxide (DMSO).

Two filter papers (12cm^2 each) were prepared on which one filter paper was impregnated with tested extract while the other filter paper was not impregnated with any extract or solvent.

The impregnated filter paper was pasted on the top of the wood rod followed by non-impregnated filter paper which was pasted below it. The same procedures were followed for the positive and negative control. Positive control used was jungle formula commercial insects' repellant and negative control was DMSO.

Twenty five *R. appendiculatus* larvae were randomly placed on a paraffin platform of the treated apparatus while the same numbers of larvae were placed on controls platform. The position of the larvae on the wood rod was recorded at 10 minutes intervals for 60 minutes. Ticks (larvae) on the impregnated filter paper and on negative control were considered not repelled while those found on non- impregnated filter paper and on wood rod were considered repelled. Three replications were done for each concentration of the extracts and standard insect repellant (Jungle formula).

Statistical analysis

Data obtained were analyzed using GenStat computer software version 4 (Gen Stat 4). Percentage repellency was calculated using the formula below as demonstrated by Silva Lima *et al.*, (2016) while effective concentration which repel 50% of the ticks (EC_{50}) was obtained using linear regression equation.

PR= NTF / (NTF +TF) X 100

Where:

PR is percentage repellent

NTF is number of ticks on non-impregnated filter paper

TF is number of ticks on impregnated filter paper.

Results

The repellency activity of *C. swynnertonii* hexane and chloroform extracts were evaluated for repellency activity against *R. appendiculatus* larvae and results obtained are summarized in Tables 1 and 2. The findings from this study indicated that the repellency activity of *C. swynnertonii* hexane and chloroform extracts was concentration and time dependent.

The repellency declined as time elapsed (Table 1 and 2). The standard repellant (Jungle formula) used displayed the same activity patterns. It was revealed that there was very high significant difference ($P<0.001$) in repellency effects between control and tested extracts in the first 40 minutes.

Hexane and chloroform extracts had comparable repellency activity as indicated in Table 1. It was evident statistically that there was nonsignificant difference ($P>0.001$) among extracts after 50 and 60 minutes exposure time (Table 1). Furthermore results revealed that, there was very high significant difference ($P<0.001$) in repellency concentrations of control and tested extracts from 10 to 60 minutes.

The concentrations of chloroform and hexane extracts with higher percentage repellency activity comparable to the control were 110mg/ml and 100 mg/ml (Table 1). Additionally, findings from this study indicated that there was very high significant interaction effect ($P<0.001$) in both control and treated extracts against concentrations in the first 20 minutes while no significant interaction effect ($P>0.001$) was observed from 30 to 60 minutes.

The concentrations that can repel 50% of the larvae EC_{50} was calculated and results are shown in Table 2. The EC_{50} was generally increasing with an increase in exposure time for both extracts and standard. The EC_{50} could not be calculated at the 10th minutes because all the tested concentrations of the standard repellant had 100% repellency while at the 20th minutes the Y-intercept was greater than 50.

Table 1. Mean percentage repellency of chloroform and hexane *C. swynnertonii* extract against *R. appendiculatus* larvae.

Extract	Concentration (mg/ml)	PR (10 min)	PR (20 min)	PR (30min)	PR (40min)	PR (50 min)	PR (60 min)
Chloroform	50	53.63de	44.84hi	24.04ij	10.89j	8.00i	6.72h
	60	58.00bcde	56.06fgh	41.44fghij	32.36ghij	22.27efghi	22.02efgh
	70	61.36bcde	61.94fg	54.04defg	41.58efgh	26.26efghi	23.18efgh
	80	71.45bc	67.13ef	50.40efgh	43.21defh	41.36cdef	27.11efg
	90	73.06b	75.88de	62.36cdeg	55.81cdefg	47.63bcde	36.69def
	100	89.00a	90.60abc	84.07abc	65.36bcde	63.41bcd	54.04bcd
	110	95.41a	92.81ab	87.10ab	74.87bc	71.17ab	57.79bc
Hexane	50	26.34f	21.21j	19.06j	14.65ij	11.06hi	7.32h
	60	47.72e	35.47i	28.26hij	24.51hij	13.71ghi	13.51gh
	70	53.03de	42.93i	39.37ghij	25.23hij	19.93fghi	17.53fgh
	80	55.97cde	46.43hi	41.49fghij	37.48fghi	27.45defgi	20.83efgh
	90	68.61bcd	54.43gh	45.13fghi	40.58fgh	37.96defg	35.04ef
	100	87.85a	77.60de	73.83bce	66.52bcd	65.72abc	56.64bc
	110	94.97a	92.26ab	75.79bc	65.55bcde	70.88ab	62.32b
Control	50	100.00a	76.77de	63.70cdef	25.13hij	23.94defgi	21.89efgh
	60	100.00a	79.66cd	60.49cdeg	42.69defh	30.45defgi	26.39efgh
	70	100.00a	80.42cd	60.65cdeg	56.66cdefg	30.35defgi	32.79efg
	80	100.00a	87.08bcd	76.63bcd	57.24cdef	36.64defh	27.80efg
	90	100.00a	100.00a	89.81a	69.12bc	46.62bcde	29.84efg
	100	100.00a	100.00a	100.00a	88.44ab	49.09bcd	40.54cde
	110	100.00a	100.00a	100.00a	100.00a	88.19a	80.41a
P-value (extracts)		***	***	***	***	ns	ns
P-value (concentration)		***	***	***	***	***	***
P-value (interaction)		***	***	ns	ns	ns	ns

***Significant difference, ns=no significant difference, values followed by different letters denote statistical significance (P<0.001).

Table 2. Effective concentrations (EC_{50}) of chloroform, hexane and control against *R. appendiculatus* larvae at different time intervals.

Extracts		Time (Minutes)					
		10	20	30	40	50	60
chloroform	EC_{50}	49.21	55.45	72.44	83.81	89.54	101.22
	R^2	0.9545	0.9785	0.9376	0.9621	0.988	0.9455
Hexane	EC_{50}	68.8	77.37	84.05	91.97	93.71	100.36
	R^2	0.9588	0.9438	0.9206	0.9227	0.9146	0.92
Control	EC_{50}	-	-	42.92	69.13	87.26	97.98
	R^2	-	0.8909	0.8777	0.9704	0.7707	0.6629

R^2 - Regression correlation coefficient; EC_{50} – Concentrations (mg/ml) at which 50% of the *R. appendiculatus* larvae were repelled.

Discussion

Repellency is caused by the release of volatile secondary metabolites which are known to cause disorders in the movement of the target species away from the odor source (Jaenson *et al.*, 2005). Plants with insecticidal repellency effects were the main insecticidal agents in Africa for management of insect vectors before the introduction of synthetic insecticidal agents. Validation of such plants has been viewed as the best option of developing bio-pesticides that are readily available in developing countries.

The *Commiphora swynnertonii* exudate is widely used by pastoralists for the management of ticks. It has therefore ethnomedical and ethnoveterinary significance in Tanzania which necessitated scientific validation of the exudate. Results emanated from this study demonstrated that hexane and chloroform extracts of *C. swynnertonii* exudate has repellency activity against *R. appendiculatus* larvae. Both *C. swynnertonii* chloroform and hexane extracts exhibited repellency activity in all concentrations (50mg/ml, 60mg/ml, 70mg/ml, 80mg/ml, 90mg/ml, 100mg/ml and 110mg/ml) which indicated that the

activity was caused by release of volatile compounds that cause the targeted organism to move against the odor source. This study confirms that the *Commiphora* species contain volatile substances that are responsible for the repellency effect that can be extracted with different organic solvents.

Findings from this paper were comparable to the results obtained from standard insects' repellant (Jungle formula). The observation was supported by Lwande *et al*., (1999) findings who reported that the essential oil of *Gynandropsis gynadra* was compared to the commercially synthetic arthropod repellant in repelling *Rhipicephalus appendiculatus* ticks. These findings clearly suggest that some plant species may contain anti-tick agents that may be equally effective against ticks as some of the commercially synthetic arthropod repellants.

The effectiveness of the extract against *R. appendiculatus* larvae was dose dependent as evidenced by an increase in percentage repellency as the concentration increased from 50mg/ml to 110mg/ml. The lower concentrations appeared to be less effective since the amount of volatile compound which enhances the repellency activity was lower than in higher concentration.

However the effectiveness of the extracts against *R. appendiculatus* was decreased as time declined from 10 to 60 minutes. EC_{50} appeared to be increasing as the time increases for both tested extracts and the standard insects' repellant. The lowest EC_{50} was appeared to be the best concentration in repelling the ticks since it induces high repellency effect within a short period of time and also minimize the utilization of plant products and hence conservation of *C. swynnertonii* diversity.

Findings from the current study on the repellency properties exhibited by *C. swynnertonii* exudates contribute to the body of knowledge on the insecticidal properties of the genus *Commiphora*. Furthermore findings from this study validate the ethnoveterinary information on the use of *C. swynnertonii* exudate on the management of tick infestation on livestock among Maasai community in Tanzania.

Conclusion

The use of plant-based products, particularly those from indigenous plants, may minimize the costs incurred by the countries to import synthetic acaricides. The results from this study strengthen the view that *C. swynnertonii* is the potential source of anti-tick agents and this validates the ethno veterinary use of the plant for management of tick infestation on the animals. The results of this study, further strengthens the widely held view that plant products can be used as an alternative to synthetic tick-repellent.

Acknowledgement

The authors wish to acknowledge the RUFORUM through RUFORUM CAA RU 2015 GRG-127 project for financial support towards the findings of this work. Authors would like to thank Philipo Mashamba, Innocent Mboya and Ibrahim for their kind help to accomplish this study.

References

Aliyu R, Catsing D, Umar HS. 2002. Antimicrobial activity and phytochemical screening of the leaves of *Commiphora africana*. Journal of Biological Sciences **13,** 75-80.

Bissinger BW, Roe RM. 2010. Tick repellents: Past, present, and future. Pesticide Biochemistry and Physiology **96,** 63-79.

Carroll JR. 1998. Kairomonal activity of white-tailed deer metatarsal gland substances: A more sensitive behavioral bioassay using *Ixodes scapularis* (Acari: Ixodidae). Journal of Medical Entomology **35,** 90-93.

Jaenson TGT, Palsson K, Borg-Karlson AK. 2005. Evaluation of extracts and oils of tick-repellent plants from Sweden. Medical and Veterinary Entomology **19,** 345-352.

Kalala W, Magadula J, Mdegela H. 2014. Evaluating Acaricidal Activity of *Commiphora swynertonii* Burrt. bark Exudate against common Ticks in Tanzania. International Journal of Herbal Medicine **2,** 19-25.

Kalala WM, Magadula JJ, Mdegela RH. 2014. Ethnobotanical use of *Commiphora swynertonii* Burrt. amongst Dorobo people in Tanzania. Journal of Medicinal Plant Research **8,** 820-828.

Lwande W, Ndakala AJ, Hassanali A, Moreka L, Nyandat E, Ndunga M, Amiani H, Gitu PM, Malonza MM, Punyua DK. 1999. Gynandropsis gynadra essential oil and its constituents as tick (*Rhipicephalus appendiculatus*) repellents. Phytochemistry **50,** 4012-4015.

Magano SR, Mkolo MN, Shai LJ. 2011. Repellent properties of *Nicotiana tabacum* and *Eucalyptus globoidea* against adults of *Hyalomma marginatum* rufipes. African Journal of Microbiology Research **5,** 4800-4804.

Mkolo MN, Magano SR. 2007. Repellent effects of the essential oil of *Lavendula angustifolia* against adults of *Hyalomma marginatum rufipes*. Journal of the South African Veterinary Association **78,** 149-152.

Novelino MAS, Daemon E, Soares GLG. 2007. Avaliação daatividade repelente do timol, mentol, salicilato de metila e ácido salicilico sobre larvas de *Boophilus microplus* (Canestrini, 1887) (Acari: Ixodidae). Arquivo Brasileiro de Medicina Veterinária e Zootecnia **59,** 700-704.

Opdebeeck JP, Wong JY, Jackson LA, Dobson C. 1988. Hereford cattle immunized and protected against *Boophilus microplus* with soluble and membrane-associated antigens from the midgut of ticks. Parasite Immunology **10,** 405-410.

Opiro R, Osinde C, Okello-onen J, Akol AM. 2013. Tick-repellent properties of four plant species against *Rhipicephalus appendiculatus* Neumann (Acarina : Ixodidae) tick species **3,** 17-21.

Silva Lima D, Carva Lho JFD, Peixoto M, Blank A, Borges LM, Costa Junior L. 2016. Assessment of the repellent effect of Lippia alba essential oil and major monoterpenes on the cattle tick *Rhipicephalus microplus*. Medica and Veterinary Entemology **30,** 73-77.

Wanzala W, Hassanali A, Mukabana WR, Takken W. 2014. Repellent Activities of Essential Oils of Some Plants Used Traditionally to Control the Brown Ear Tick, *Rhipicephalus appendiculatus*. Journal of Parasitology Research **2014,** 10 pages.

Ambient Science, 2017: Vol. 04(2); 11-16
DOI:10.21276/ambi.2017.04.2.ra02

Published by: National Cave Research and Protection Organization, India

ambient SCIENCE
Vol. 04(2): 11-16
Year 2017

RESEARCH ARTICLE

Acute and Sub-acute Toxicity of *Commiphora swynnertonii* extracts: an Experimental Study on Albino Mice and Rats

Disela Edwin[1], Paul Erasto[2], Sylvester Temba[1], Musa Chacha[1]*

[1]School of Life Sciences and Bio-Engineering, Nelson Mandela African Institution of Science and Technology, P.O. Box 447, Arusha-Tanzania

[2]National Institute of Medical Research, P.O. Box 9653 Dar es salaam-Tanzania

Study Area: Simanjiro district in Arusha region, Tanzania

Coordinates: 3"34'S; 3"659'E

Key words: Hematological, Biochemical, Histology, Body- weight.

Abstract

The acute and subacute toxicity of *Commiphora swynnertonii* extracts on albino mice and rats was verified. For the acute toxicity study, oral administration of *C. swynnertonii* hexane and chloroform extract with doses of 500, 1000, and 2000 mg/kg body weight induced no treatment-related signs of toxicity in the animals during the 14 days of the experimental period. In the sub-acute toxicity, *C. swynnertonii* hexane extract showed a significant decrease in mean corpuscular volume (MCV), hematocrit (HCT) and a significant increase in Mean platelet volume (MPV). A significant decrease in aspartate amino-transferase (AST) and creatinine was also observed. In the rats treated with *C. swynnertonii* chloroform extract, the significant change in white blood cell (WBC), Red blood cell (RBC), Mean corpuscular volume (MCV), Mean hemoglobin concentration (MCH), Mean platelet volume (MPV) and hemoglobin (Hb) were observed.The significant change in alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (ALP), creatinine, albumin and bilirubin was also observed.

Introduction:

Commiphora swynnertonii (Burseraceae) is widely distributed in the Northern part of Tanzania and Southern part of Kenya, (Kalala *et al.,* 2014) including tropical and subtropical part of Asia and South America (Adam *et al.,* 2013). It is a small tree highly branched with spine reaching a height of about 2 to 3 meters (Ruffo *et al.,* 2002).The barks are the pale grey that peels off in papery pieces and when damaged it release a watery, milky sap which later becomes reddish brown resinous exudates. The sap of *C. swynnertonii* has for a long time been utilized as anti-ectoparacites and is applied on animal skins for control of ticks, fleas, tsetse flies, bed bugs and mange mites (Kaoneka *et al.,* 2007). A number of investigations have focused on the validation of ethnoveterinary and ethnomedical information of *C. swynnertonii* plant. Sambuta & Masola (2006) reported the anti-ectoparasitic effects of *C. swynnertonii* extracts against ticks, fleas and mites. Mkangara *et al.*,20154a reported acaricidal activity of C. swynnertonii stem bark extracts against *Rhipicephalus appendiculatus* and *Amblyomma variegatum*. *C. swynnertonii* were reported to exhibit antimicrobial, antifungal and antiviral activity (Mkangara *et al.,* 2014b). This paper reports the acute and subacute toxicity of *C. swynnertonii* hexane and chloroform extracts.

Methodology:

Exudates collection: exudates of *C. swynnertonii* were collected from Mererani, Simanjiro district in Arusha region, Tanzania. The plant was identified by a botanist from TPRI and voucher specimen coded CS 001 is deposited at the Nelson Mandela African Institution of Science and Technology.

Extraction of plant materials: exudates weighed 78.8 g was mixed with 150 ml of distilled water followed by 150 ml of hexane. The solution was thoroughly shaken and allowed to settle in order to form two layers. The hexane layer was separated from aqueous layer by decantation. Further, 150 ml of chloroform was poured into the separating funnel containing aqueous layer. The solution was shaken and left for 6 hrs and the chloroform layer was separated from the aqueous layer. The two extracts obtained were concentrated through vacuum rotary evaporator, the extracts obtained were kept in a beaker covered with aluminum foil, stored at 4°C for further use.

*Corresponding Author: musa.chacha@nm-aist.ac.tz

ISSN- 2348 5191 (Print) & 2348 8980 (Electronic)

Ambient Science, 2017: Vol. 04(2); 11-16
DOI:10.21276/ambi.2017.04.2.ra02

Experimental animals: albino mice of both sexes, weighted between 19-36 g aged 4 to 5 weeks and albino rats of both sexes weighed between 46-192g aged 7 - 8 weeks were randomly obtained from Sokoine University of Agriculture (SUA), Morogoro, Tanzania. The animals were allowed to stay in cages with sawdust litters at a controlled temperature of about 25°C to 29°C and lighting of 12 hours of light and 12 hours of darkness for each 24 hours period.

Acute oral toxicity: the toxicity study was done according to Organization for Economic Co-operation and Development (OECD) guidelines number 425 of 2001 (OECD/OCDE, 2001). The mice were acclimatized for 7 days before treatments, and they were provided with standard diet and water. The animals were divided into seven groups of six animals each (3 males and 3 females), one control groups and six treated groups. Body weights of the mice were determined and the dose was calculated in accordance with their body weights. The control group was given distilled water and the three treated groups were given doses of 500, 1000 and 2000 mg/kg body weight of hexane extract while other three groups were given doses of 500, 1000 and 2000 mg/kg body weight of chloroform extract. The animals were observed for their toxic symptoms, behavioral changes, and mortality at least once daily for 14 days.

Sub-acute toxicity: forty-two albino rats were divided into seven groups of six animals each (3 males and 3 females), one control groups and six treated groups. Control group was given distilled water and the three treated groups were given doses of 250, 500 and 1000 mg/kg body weight of hexane extract while other three groups were given doses of 250, 500 and 1000 mg/kg body weight of chloroform extract. All administrations were done every 24 hours via oral gavage throughout the experimental period. The rats were weighed daily and also subjected to thorough observations for mortality and any behavioral changes, during the 28-day experimental period. After 28 days all rats were weighed and the blood of each rat was collected through eye orbital vein into two vacutainer tubes for each animal. Hematological parameters including white blood cell (WBC), Red blood cell (RBC), Hematocrit (HCT), Mean corpuscular volume (MCV), Mean hemoglobin concentration (MCH), Mean platelet volume (MPV), Mean corpuscular hemoglobin concentration (MCHC), and hemoglobin (Hb) were determined using the blood samples contained in the EDTA tubes. The blood samples contained in plain vacutainer tubes were centrifuged and the serum obtained was subjected to biochemical parameters such as total protein, bilirubin, alkaline phosphate (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine and albumin. After blood collection, all rats were dissected and internal organs such as liver, heart, lungs, spleen, and kidneys were carefully removed and weighed individually. Organs were fixed in 10% formalin then sections were prepared and stained with hematoxylin and eosin before microscopic examination.

Statistical analysis: the Student's t-test was used to compare initial body weight and final body weight for both control and treated groups of animals while one-way analysis of variance (ANOVA) was used for multiple comparisons of the means for hematological, biochemical and organ weight data between the control and treated groups of animals. All statistical analysis was by STATISTICA software-8 ; level of significance $p < 0.05$.

Results :

Acute oral toxicity: the oral administration of *C. swynnertonii* hexane and chloroform extract at 500, 1000 and 2000 mg/kg body weight doses had no clinical adverse effect of substance related toxicity and did not cause any mortality during the 14-days observation period.

Sub-acute toxicity: daily repeated oral administration of *C. swynnertonii* hexane and chloroform extract at 250, 500 and 1000 mg/kg body weight doses for 28 days did not induce any evident sign of toxicity and mortality in the treated animals.

Body weight changes: in the *C. swynnertonii* hexane extract the significant increase ($p<0.05$) in body weight of male rats was observed in control and at the dose of 250 mg/kg body weight (Table-1). In *C. swynnertonii* chloroform extract the significant increase ($p<0.05$) in body weight was observed in control male while at the dose of 500 mg/kg body weight, the significant increase ($p<0.05$) in body weight was observed in both male and female rats (Table -2).

Organ weight: finding from this study revealed that the weight of the liver treated with *C. swynnertonii* hexane extract was significantly decreased ($p<0.05$) while the other organs such as heart, spleen, lung and kidney showed no significant change in weight (Table-3). Further the significant decrease ($p<0.05$) in weight of liver, heart, spleen, lung, and kidney were observed in the rats treated with *C. swynnertonii* chloroform extract (Table -4).

Hematological parameters: finding from this study revealed that, with the exception of significant decrease ($p<0.05$) in MCV, HCT and significant increase ($p<0.05$) in MPV in the rats treated with *C. swynnertonii* hexane extract, administration of this extract at all the investigated doses had no significant ($p>0.05$) effect on WBC, RBC, MCH, MCHC and Hb when compared with control (Table-5). Additionally the significant decrease ($p<0.05$) in WBC, RBC, MCV, MCH, Hb and significant increase ($p<0.05$) in MPV was observed in the rats treated with *C. swynnertonii* chloroform extract (Table-6).

Ambient Science, 2017: Vol. 04(2); 11-16
DOI:10.21276/ambi.2017.04.2.ra02

Biochemical parameters: significant decrease ($p<0.05$) in ALP level was observed in male rats whereas significant decreased in AST and creatinine was observed in the female rats treated with *C. swynnertonii* hexane extract (Table-7). Furthermore significant increase ($p<0.05$) in ALT, AST and creatinine observed in rats treated with *C. swynnertonii* chloroform extract while significant decrease ($p<0.05$) in albumin and significant increase ($p<0.05$) in bilirubin was observed in the male rats treated with *C. swynnertonii* chloroform extract (Table -8).

Histopathological analysis: the microscopic examinations of the liver, kidney, spleen, lung, and heart of the animals treated with *C. swynnertonii* hexane extract revealed no abnormalities in the overall structural orientation of the organs during subacute toxicity study (Fig.-1). Furthermore, the examination of a liver section of the animals treated with *C. swynnertonii* chloroform extract revealed moderate to severe congestion and the initial stage of necrosis (Fig.-2). The kidney section of the animals treated with *C. swynnertonii* chloroform extract induced congestion and destruction in the glomerulus's shape (Fig.-2).

Table-1: Body weight (g) of control & rats treated with *C. swynnertonii* hexane extract measured during sub-acute toxicity

Dose (mg/kg BW)	Sex	Mean±SEM at day 0	Mean±SEM at day 28	P-value
Control	M	80 ± 3.00	123 ± 8.00	0.04
	F	107 ± 13.00	117.5 ± 20.5	0.70
250	M	68 ± 5.00	104.5 ± 5.5	0.04
	F	58.5 ±12.5	82 ± 12.00	0.31
500	M	87.5 ± 26.5	96.5 ± 30.5	0.84
	F	58 ± 2.00	77.5 ± 7.5	0.13
1000	M	154.5 ± 37.5	192.5 ± 15.5	0.45
	F	115 ± 23.00	121 ± 21.00	0.86

Table-2: Body weight (g) of control and rats treated with *C. swynnertonii* chloroform extract measured during sub-acute toxicity

Dose (mg/kg BW)	Sex	Mean±SEM at day 0	Mean±SEM at day 28	P-value
Control	M	80 ± 3.00	123 ± 8	0.04
	F	107 ± 13.00	117.5 ± 20.5	0.71
250	M	72 ± 7.00	96 ± 20.00	0.37
	F	67 ± 8.00	79.5 ± 0.5	0.26
500	M	77.5 ± 1.5	104.5 ± 2.5	0.01
	F	63 ± 0.00	90.5 ± 3.5	0.01
1000	M	116.5 ± 12.5	131 ± 10.00	0.46
	F	121.5 ± 21.5	143.5 ± 20.5	0.54

M = male, F = female, BW = body weight

Table- 3 to 8: Different superscripts denote statistical significance as per Duncan's multiple range test ($p<0.05$)

Table-3: Organ-body weight of control & rats treated with *C. swynnertonii* hexane extract measured during sub-acute toxicity.

Organ	Sex	Control	250*	500*	1000*	P-value
Liver	M	3.50±0.11^{a}	2.42±0.28^{b}	3.47±0.60^{a}	3.16±0.28a	0.02
	F	4.16±0.48^{a}	4.41±0.48^{a}	3.65±0.01^{a}	1.98± 0.22^{b}	0.03
Heart	M	0.59±0.04	0.44±0.02	0.63±0.05	0.45±0.03	0.06
	F	0.59±0.02	0.58±0.11	0.43±0.06	0.38±0.10	0.3
Spleen	M	0.25±0.02	0.21±0.01	0.39±0.05	0.22±0.03	0.06
	F	0.32±0.02	0.27±0.04	0.26±0.00	0.20±0.05	0.22
Lungs	M	0.78±0.18	0.58±0.03	1.18±0.24	0.69±0.14	0.20
	F	1.06±0.25	1.09±0.24	0.74±0.12	0.44±0.14	0.12
Kidney	M	0.49±0.16	0.32±0.02	0.84±0.16	0.33±0.02	0.09
	F	0.48±0.01	0.49±0.11	0.43±0.03	0.29±0.05	0.09

Values: mean ± SEM, M=Male, F=Female, *mg/kg BW

Table-4: Organ-body weight of control and rats treated with *C. swynnertonii* chloroform extract measured during sub-acute toxicity

Organ	Sex	Control	250*	500*	1000*	P-value
Liver	M	3.50±0.11^{b}	2.92±0.21^{a}	2.88±0.05^{a}	2.79±0.04^{a}	0.04
	F	4.16±0.48^{b}	3.73±0.28^{a}	3.21±0.15a	3.50±0.17^{a}	0.02
Heart	M	0.59±0.04^{b}	0.38±0.00^{a}	0.56±0.01^{b}	0.43±0.02^{a}	0.01
	F	0.59±0.02^{b}	0.49±0.07^{ab}	0.30±0.07^{a}	0.40±0.02^{a}	0.04
Spleen	M	0.25±0.02^{a}	0.28±0.01^{a}	0.25±0.00^{ab}	0.21±0.02^{b}	0.02
	F	0.32±0.02^{b}	0.23± 0.01^{a}	0.18±0.01^{a}	0.23±0.02^{a}	0.01
Lungs	M	0.78±0.18^{c}	0.54±0.02^{b}	0.79±0.02^{c}	0.63±0.02^{a}	0.001
	F	1.06±0.25^{b}	0.95±0.00^{d}	0.85±0.00^{c}	0.74±0.00^{a}	0.001
Kidney	M	0.49±0.16^{b}	0.32±0.00^{ab}	0.29±0.01^{a}	0.36±0.01^{c}	0.011
	F	0.48±0.01^{a}	0.42±0.01^{ab}	0.49±0.05^{a}	0.36±0.00^{b}	0.04

Values: mean ± SEM, M=Male, F=Female, *mg/kg BW

Table-5: Hematological values of control and rats treated withC. swynnertonii hexane extract measured during sub-acute toxicity

Parameter	Sex	Control	250*	500*	1000*	P-value
WBC	M	56.05±0.62	59.91±3.02	52.22±0.24	48.86±0.41	0.06
(m/mm3)	F	58.43 ±0.54	48.66±4.71	51.73±1.17	48.33±0.57	0.12
RBC	M	6.83±0.67	3.61±1.01	14.37±5.05	5.81±0.31	0.08
(m/mm3)	F	6.33±0.60	5.19±2.05	5.41±0.11	4.36±0.01	0.51
MCV	M	66.15±0.05	58.65±2.65	60.94±0.16	59.6±0.90	0.06
(fl)	F	61.5±0.40^{b}	59.7±0.80^{a}	60.2±1.00^{a}	61.25±0.36^{a}	0.03
HCT(%)	M	37.2±2.70^{a}	20.85±4.95^{b}	35.75±0.15^{a}	34.55±1.35^{a}	0.04
	F	38.9±3.90	30.7±11.80	32.5±1.20	26.8±0.10	0.70
MCH	M	23.2±1.90	25.65±3.35	25.24±0.37	24.7±0.70	0.82
(pg)	F	23.85±0.15	21.8±0.80	26.7±0.70	27.24±0.37	0.06
MCHC	M	42.5±2.40	44.15±7.75	41.89±0.21	41.5±0.60	0.97
(g/dl)	F	38.9±0.10	36.7±1.80	44.4±0.40	45.06±0.16	0.05
Hb	M	15.75±0.25	9.6±3.80	15.35±0.35	14.35±0.35	0.23
(g/dl)	F	16.65±0.05	11.5±4.90	14.45±0.65	12.22±0.12	0.63
MPV	M	7.25±0.05	7.3±0.30	14.45±0.05	7.3±0.10	0.39
(fl)	F	7.5±0.00^{a}	7.3±0.00^{a}	14.45±0.05^{b}	7.5±0.00^{a}	0.021

Values: mean ± SEM, M=Male, F=Female, *mg/kg BW

Ambient Science, 2017: Vol. 04(2); 11-16
DOI:10.21276/ambi.2017.04.2.ra02

Table-6: Hematological values of control and rats treated with *C.swynnertonii* chloroform extract measured during sub-acute toxicity

Parameter	Sex	Control	250*	500*	1000*	P-value
WBC	M	56.05±0.62^{d}	53.06±0.16^{c}	51.22±0.24^{b}	47.61±0.61^{a}	0.001
(m/mm3)	F	58.43±0.54^{c}	54±0.35^{b}	51.05±1.49^{ab}	47.58±0.82^{a}	0.001
RBC	M	6.33±0.17^{c}	4.27±1.15^{a}	4.76±0.09^{b}	5.71±0.05^{a}	0.001
(m/mm3)	F	6.83±0.10^{a}	6.73±0.12a	5.39±0.44^{c}	4.60±0.96^{b}	0.001
MCV	M	66.15±0.05^{d}	62.45±0.35^{b}	63.38±0.18^{c}	60.59±0.01^{a}	0.001
(fl)	F	61.5±0.40^{a}	58.14±0.27^{a}	60.5±2.20^{a}	60.55±0.05^{b}	0.01
HCT(%)	M	37.2±2.70	32.3±1.60	30.85±0.24	34.93±0.13	0.14
	F	38.9±3.90	38.80±0.20	26.35±7.95	27.8±5.80	0.31
MCH	M	25.1±0.1^{b}	24.6±0.50^{ab}	23.63±0.07^{a}	24.14±0.06^{a}	0.05
(pg)	F	23.85±0.15^{ab}	21.93±0.37^{a}	21.9±1.30^{a}	27.3±1.30^{b}	0.04
MCHC	M	42.5±2.40	36.35±2.75	37.55±0.05	39.10±0.20	0.23
(g/dl)	F	38.9±0.10	38.6±0.30	36.5±3.40	45.2±2.10	0.13
Hb	M	15.75±0.25^{c}	12.95±0.65^{b}	11.45±0.05^{a}	10.5±0.20^{a}	0.002
(g/dl)	F	16.65±0.05^{d}	14.5±0.30^{c}	13.4±0.30^{b}	9.7±0.20^{a}	0.001
MPV	M	7.25±0.05^{b}	7.65±0.05^{a}	7.65±0.05^{a}	7.55±0.05^{a}	0.01
(fl)	F	7.5±0.00	7.6±0.00	7.5±0.10	7.35±0.15	0.39

Table-7: Biochemical parameters of control and rats treated with *C. swynnertonii* hexane extract measured during sub-acute toxicity

Parameter	Sex	Control	250*	500*	1000*	P-value
ALT	M	46.66±3.33	45.00±1.67	46.66±0.00	46.66±3.33	0.95
(u/l)	F	54.99±1.67	54.99±8.33	49.99±0.01	69.99±0.00	0.10
AST	M	88.32±8.33	88.32±31.66	119.99±0.00	63.33±10.00	0.28
(u/l)	F	84.99±1.67^{d}	76.66± 3.33^{c}	53.33±3.33^{a}	66.67±0.01^{b}	0.01
ALP	M	262.58±135.44	253.686±3.37	250.41±0.09	210.188±0.12	0.94
(u/l)	F	323.39 ±66.34	187.95±96.74	100.89±17.97	69.99±0.00	0.12
Albumin	M	3.94±0.05	3.00±0.78	3.40±0.00	3.04±0.03	0.40
(g/dl)	F	3.71±0.02	4.15±0.60	3.79±0.65	2.61±0.01	0.22
Bilirubin	M	1.24±0.32	1.08±0.12	0.97±0.01	1.07±0.49	0.93
(mol/l)	F	1.24±0.60	0.79±0.77	0.66±0.08	0.62±0.00	0.8
T.protein	M	7.16±0.11	7.61±0.76	8.74±0.00	7.00±0.64	0.20
(g/dl)	F	7.14±0.49	6.52±0.44	6.12±0.53	6.91±0.01	0.44
Creatinine	M	0.67±0.08	0.67±0.08	0.58±0.00	0.62±0.04	0.74
	F	0.54±0.04^{a}	0.49±0.05^{a}	0.54±0.04^{a}	0.33±0.00^{b}	0.05

Table-8: Biochemical parameters of control and rats treated with *C. swynnertonii* chloroform extract measured during sub-acute toxicity

Parameter	Sex	Control	250*	500*	1000*	P-value
ALT	M	46.66±3.33^{a}	54.99±8.33^{a}	82.82±0.51^{b}	59.99±0.01^{a}	0.018
(u/l)	F	54.99±1.67^{a}	43.33±0.00^{b}	58.33±1.67^{a}	68.33±1.67^{c}	0.001
AST	M	88.32±8.33	81.66±1.67	89.44±0.56	90.17±0.51	0.54
(u/l)	F	84.99±1.67^{a}	149.99±0.00^{c}	96.66±3.34^{b}	87.60±0.50^{a}	0.001
ALP	M	262.58±135.44	382.81±134.05	391.99±0.50	425.66±0.00	0.67
(u/l)	F	323.39 ±66.34^{b}	304.04±0.00^{b}	152.02±11.06^{a}	149.26±2.76^{a}	0.037
Albumin	M	3.94±0.05^{a}	3.81±0.25^{a}	3.55±0.00^{a}	4.70±0.01^{b}	0.011
(g/dl)	F	3.71±0.02	3.29±0.00	3.85±0.30	3.95±0.01	0.12
Bilirubin	M	1.24±0.32^{b}	2.53±0.07^{a}	3.09±0.15^{a}	3.57±0.42^{a}	0.01
(mol/l)	F	1.24±0.60	1.26±0.50	2.10±0.28	2.59±0.10	0.19
T.protein	M	7.16±0.11	8.51±0.95	7.19±0.01	7.83±0.01	0.29
(g/dl)	F	7.14±0.49	7.67±0.00	7.68±0.10	8.12±0.22	0.24
Creatinine	M	0.67±0.08	0.79±0.04	0.82±0.00	0.94±0.02	0.294
	F	0.54±0.04^{b}	0.83±0.00^{a}	0.85±0.00^{a}	0.92±0.03^{a}	0.002

Values: mean ± SEM, M=Male, F=Female, *mg/kg BW

Discussion:

The acute toxicity was investigated in order to determine whether administration of a single high dose of *C. swynnertonii* hexane and chloroform extract brought any adverse effect on tested animals during the 14 days after the oral administration. In the present study, oral administration of *C. swynnertonii* hexane and chloroform in albino mice at 500, 1000 and 2000 mg/kg for 14 days had no effects on mortality or any examined clinical signs of toxicity. No acute toxicity was found in mice treated with *C. swynnertonii* extracts, so the approximate lethal dose was determined to be higher than 2000 mg/kg.

The sub-acute toxicity study, which involved rats given *C. swynnertonii* hexane and chloroform extract at doses of 250, 500 and 1000 mg/kg body weight, generally elicited no clinical signs of toxicity, mortality or any behavioral changes.The results from this study indicated that *C. swynnertonii* hexane and chloroform extract induced a normal change in body weight and therefore it can be suggested that the tested extracts had no effects on the body weights of the rats.

Organ weight is an important index to detect whether the organ was exposed to the injury or not (Jothy *et al.*, 2011). Findings from this study revealed that the observed significant decreased in the weight of the liver of rats treated with *C. swynnertonii* hexane extract was not corroborated by both the histological examination and other biochemical parameters of liver function and therefore indicating that the extract is virtually nontoxic. The significant decrease in liver, heart, spleen, lung, and kidney weight of rats treated with *C. swynnertonii* chloroform extract indicating that chloroform extract contains some toxic substances that affected the weight of internal organs of the rats. Blood is an important index of physiological status in both man and animals (Vaghasiya *et al.*, 2011). In this study, the nonsignificant difference in WBC, RBC, MCH, MCHC and Hb of the rats' treatment with *C. swynnertonii* hexane extract indicated that the extract may be nontoxic to the blood. Reductions in RBC, MCV, MCH and Hb of the rats treated with C. swynnertonii chloroform extract indicated that the extract interfered with the normal production of hemoglobin and its concentrations within red blood cells. Thus, it can be suggested that *C. swynnertonii* chloroform extract may possess the

Ambient Science, 2017: Vol. 04(2); 11-16
DOI:10.21276/ambi.2017.04.2.ra02

Figure-1(a&b): Treatment effects on histological examinations of the main organs in the rats during subacute toxicity study. Representative photomicrographs from liver, kidney, spleen, lung and heart sections stained with hematoxylin and eosin (×40), respective groups: (A) control group (B) treated group (250mg/kg) (C) treated group (500mg/kg) (D) treated group (1000mg/kg).

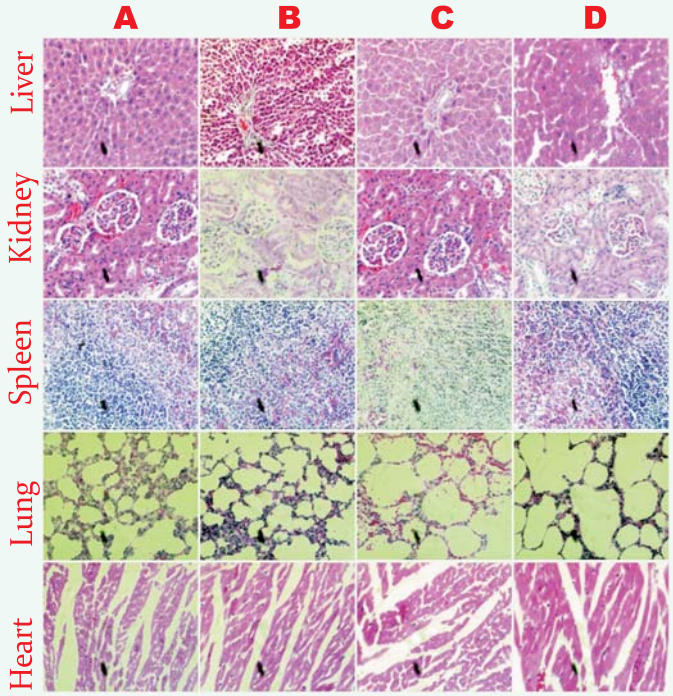


Figure-1a: Treatment- *C. swynnertonii* hexane extract

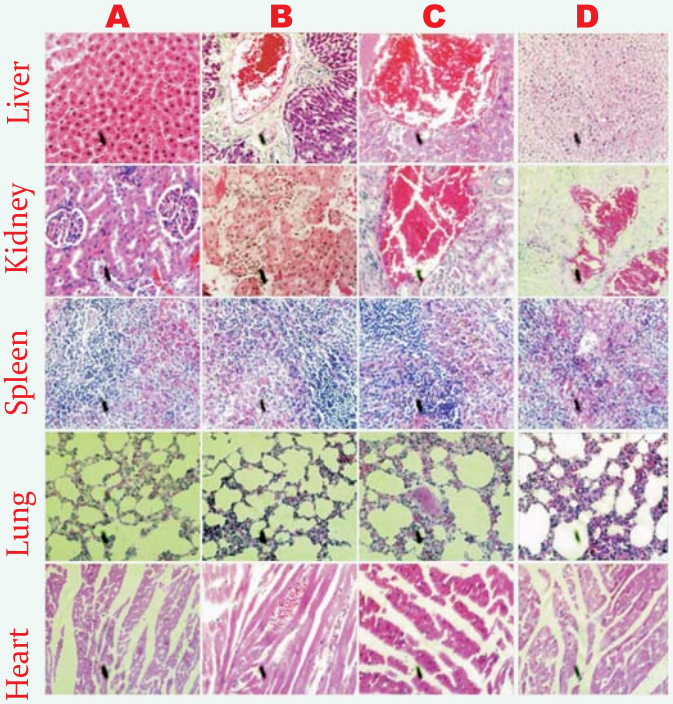


Figure-1b: Treatment- *C. swynnertonii* chloroform extract

potential to induce anemia (Amna *et al.,* 2013). The observed reduction in WBC in the rats treated with *C. swynnertonii* chloroform extract may suggest a decline in the function of the immune system. Evaluation of biochemical parameters may provide useful information regarding the overall health status and alteration in metabolic processes of the animals caused by ingestion of plant extracts (Tarkang *et al.,* 2012). The liver and the kidneys are target organs for toxic chemicals due to their essential functions in biological detoxication and excretion processes (Bello *et al.,* 2016). When the liver cell is damaged, a variety of enzymes normally located in the cytosol are released into the blood stream. The transaminases (AST and ALT) and ALP are well-known enzymes used as good indicators of liver function (Ismail *et al.*, 2014). The kidneys excrete metabolic waste products and regulate the serum concentration of a variety of substances (Benouadah *et al.*, 2016). Creatinine is an important biomarker of renal toxicity and increases the levels of these parameters indicates a marked renal damage (Suryavanshi *et al.*, 2015). Results from this study showed that oral administration of *C. swynnertonii* hexane extract to albino rats revealed the insignificant changes of most of the serum biochemical parameters which suggest the non-toxic nature of the extract. The observed significant increase in ALT, AST and creatinine for female; albumin and bilirubin for the male in the rats treated with *C. swynnertonii* chloroform extract indicated that the extract may induce liver damage probably by altered cell membrane permeability leading to the leakage of the enzymes from the tissues to the serum. The histopathological result of liver sections of rats treated with *C. swynnertonii* chloroform extract confirmed these effects indicating normal to severe congestion and the initial stage of necrosis. Regarding the kidney function, the significant increase in serum level of creatinine in the test groups treated with *C. swynnertonii* chloroform extract suggests possible renal damage. Histological aspects indicated a severe congestion and destruction of glomerular's shape in the kidney.

Conclusion:

The results from acute toxicity concluded that LD50 of *C. swynnertonii* hexane and chloroform extracts is above 2000 mg/kg body weight. Following its 28-day repeated daily oral dose administration of *C. swynnertonii* hexane and chloroform extracts in the animals, it may be concluded that *C. swynnertonii* hexane extract does not elicit any treatment-related adverse effect at the investigated doses and thus may be classified to be virtually non-toxic. The observed some changes in the hematological, serum biochemical parameters with end-organ damage to the liver and kidney which lead to the alterations in the normal physiological functions of the animals, it can be concluded that the prolonged

consumption of *C. swynnertonii* chloroform extract can cause some virtually toxic effect in the blood and also cause some damage to the internal organs. Therefore, caution and safety measures should be taken before oral ingestion of *C. swynnertonii* chloroform extract.

Acknowledgements:

This work is sponsored by Regional Universities FORUM for capacity building in agriculture. The authors wish to thank RUFORUM through RUFORUM CAA Project for financial support towards preparation of this work.

References:

Adam, M.E. & Selim S.A. (2013): Antimicrobial activity of essential oil and methanol extract from *Commiphora molmol* (Engl.) resin. *Int. J. Curr. Microbiol. App. Sci.*, 2(12): 1-6.

Amna, O.F., Nooraain, H., Noriham, A., Azizah, A. & Husna R.N. (2013): Acute and oral subacute toxicity study of ethanolic extract of *Cosmos caudatus* leaf in Sprague Dawley rats. *Int. J. Bios. Biochem. Bioinform.*, 3(4): 301-305.

Bello, I., Bakkouri, A.S, Tabana, Y.M. & Al-hindi, B. (2016): Acute and Sub-Acute Toxicity Evaluation of the Methanolic Extract of *Alstonia scholaris* Stem Bark. *Med. Sci.*, 4(1): 4 .

Benouadah, Z., Mahdeb, N., Bouzidi, A. (2016): Evaluation of Acute and Sub-Acute Toxicity of Alkaloids from *Datura stramonium* Sp. in Mice. *Int. J. Pharmacog. Phytochem. Res.*, 8: 1759-1766..

Ismail, Z., Halim, S.Z., Abdullah, N.R., Afzan, A., Abdul Rashid, B.A. & Jantan, I. (2014): Safety Evaluation of Oral Toxicity of Carica papaya Linn. leaves: A Subchronic Toxicity Study in Sprague Dawley Rats. *Evi-Based Compl. Alt. Med.*, Article ID 741-470.

Jothy, L.S., Zakaria, Z., Chen. Y., Lau, Y.L., Yoga Latha, L. & Sasidharan, S. (2011): Acute oral toxicity of methanolic seed extract of *Cassia fistula* in mice. *Molecules.* 16(6); 5268-5282.

Kalala, W., Magadula, J., & Mdegela, H. (2014): Evaluating Acaricidal Activity of *Commiphora swynertonii* (Burrt .) bark Exudate against common Ticks in Tanzania. *Int. J. Herb. Med*., 2(4): 19-25.

Kaoneka, B., Mollel, M. & Lyatuu, F. (2007): Leaf essential oil composition and tick repellency activity of *Commiphora swynnertonii* Burtt. *J. Biol. Res.*, 8: 213-216 .

Mkangara, M., Erasto, P. & Chacha, M. (2014a): Acaricidal activity of *Commiphora Swynnertonii* (Burtt) stem bark extracts against adult *Rhipicephalus Appendiculatus* newman and *Amblyomma Variegatum*. *Am. J. Res. Comm.*, 2(9): 82-92.

Mkangara, M., Chacha, M. & Kazyoba, P.E. (2014b): Antimicrobial and Cytotoxicity Efficacy of *Commiphora swynnertonii* (Burtt) Extracts. *Int. J. Sci. Res.*, 3(7): 1611-1615 .

OECD/OCDE (2001): Acute oral toxicity- up-and down procedure. **OECD Guidelines for testing of chemicals.**No. 425 . P. 1-26.

Sambuta, A.K. & Masola, S.N. (2006): The efficacy of *Commiphora swynnertonii* extracts in the control of external parasites in livestock. **Proc. Papers COSTECH**, 42 pages.

Suryavanshi, S.A., Shinde-Kadam, K., Raina, P., Nimbargi, R., Pandit, V.A. & Kaul-Ghankar, R. (2015): Evaluation of Acute and Sub-Acute Toxicity of a Standardized Polyherbal Formulation (HC9): An In Vivo Study. *Int. J. Pharm. Pharmaceut. Sci.*, 7(11): 110-117 .

Tarkang, P.A., Agbor, G.A., Armelle, T.D., Yamthe, T.L.R., David, K. & Ngadena, Y.S.M. (2012): Acute and Chronic Toxicity Studies of the aqueous and ethanol leaf extracts of Carica papaya Linn in Wistar rats. *J. Nat. Prod.*, 2(5):617627.

Vaghasiya, Y., Shukla, V. & Chanda, S. (2011): Acute Oral Toxicity Study of *Pluchea arguta* Boiss Extract in mice. *J. Pharmacol. Toxicol.*, 6(2), 113-23 .

RUFORUM Working Document Series (ISSN 1607-9345) No. 14 (1): 799-802.
Available from http://repository.ruforum.org

Research Application Summary

Contribution of natural products in the management of livestock diseases

Swai, D.[1], Erasto, P.[2] & Chacha, M.[1]
[1]School of Life Science and Engineering, Nelson Mandela African Institution of Science and Technology, P. O. Box 447, Arusha, Tanzania
[2]National Institute for Medical Research (NIMR), P. O. Box 9653, Dar es Salaam, Tanzania
Corresponding author: edwind@nm-aist.ac.tz

Abstract

Before the introduction of synthetic veterinary drugs (SDs), plants were used as the basis of traditional remedy for the management of livestock diseases in Africa. Despite the fact that most of the commercially SDs are effective, smallholder farmers especially those living in remote rural areas are not easily accessing them. They therefore rely on the use of indigenous plants which are known to be effective in the management of livestock diseases. The knowledge on the use of these plants have been well preserved in societies and verbally transmitted generations to generations. This paper is therefore presenting a review on plants of ethno veterinary relevance and bioactive compounds report there from.

Key words: Bioactive compounds, ethno veterinary, veterinary drugs

Résumé

Avant l'introduction de médicaments vétérinaires synthétiques (MSs), les plantes étaient utilisées comme base de remède traditionnel pour la gestion des maladies animales en Afrique. Malgré le fait que la plupart des MSs modernes sont efficaces, les petits agriculteurs en particulier ceux qui vivent dans les zones rurales reculées n'en ont pas accès facile. Ils dépendent donc de l'utilisation des plantes indigènes qui sont connues pour être efficace dans la gestion des maladies animales. Les connaissances sur l'utilisation de ces plantes ont été bien conservées dans la société et verbalement transmises de générations en générations. Ce document présente donc un examen sur les plantes de rapport de pertinence vétérinaire et les composés bioactifs ethno là depuis.

Mots clés: composés bioactifs, vétérinaires ethno, les médicaments vétérinaires

Background

Plants have contributed a great deal in the development of drug leads that were eventually developed into drugs for the management of human diseases (Jasuja *et al*., 2012). It therefore justifies the relevance of ethnomedical information that has been passed on from

generations to generation in many societies in the world. Unlike ethnomedical information which has contributed immensely in the development of clinically useful drugs, only a limited number of plants used for the management of livestock disease have been exploited for the same. Unlike synthetic veterinary drugs where some of them are posing some side effects to animals, plants which are traditionally used for the management of livestock diseases since time immemorial, are known be compatible with other biological systems. The recently established resistance of livestock disease vectors and pathogens has provided the momentum for the need to discover new classes of veterinary drugs with unique mechanism of actions. One of the best source so far is nature which has never stopped supporting human, livestock as well as wild animals (Makkar *et al.*, 2009). Plants and compounds therefrom with veterinary relevance are discussed below.

Method

Scientific papers published in the period of 2003 to 2015 which report compounds with insecticidal and anthelmintic activity were reviewed. The focus was also on compounds that either induce mortality or deter ticks and other insects of veterinary relevance. Papers were internet sourced.

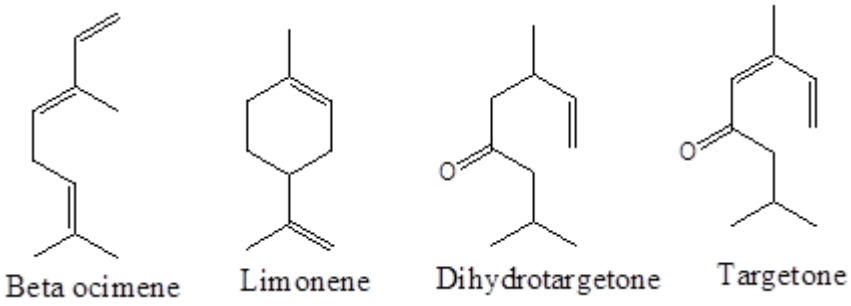
Results

The use of plants for the management of livestock diseases is practiced not only in Africa but also in other parts of the world. For instance, *Tagetes minuta* which is an annual perennial herb native to temperate grassland and montane region of South America, and exotic species to Africa, Europe, Asia and Australia is used by communities from these countries for the management of ticks (Hulina, 2008; Ofori *et al*., 2013) The acaricidal activity of this plant was established to be exerted by the essential oil. *Tagetes minuta* essential oil (TMEO) was found to induce acaricidal activity to both hard and soft ticks. Garcia and Coworkers (2012) reported that TMEO contains terpenes namely limonene (6.96%), β-ocimene (5.11%), dihydrotagetone (54.21%) and tagetone (6.73%).

Terminalia arjuna growing on river banks or near river beds in South and Central India was reported to exhibit anthelmintic activity against *Haemonchus contortus* and gastrointestinal trichostrongyled nematodes in sheep (Bachaya *et al*., 2009). The anthelmintic activity was due to presence of condensed tannin which may impair vital processes such as feeding and reproduction of the parasites or may bind and disrupt the integrity of the parasites' cuticle. However the anthelmintic effect of tannin may be attributed to its ability to bind free protein available in the tubes for larva nutrition. This reduces availability of nutrient which result in larva starvation or decrease in gastrointestinal metabolism through destruction of metabolic pathway of nutrient oxidation. This results in no release of energy which is in form of ATP (Bachaya *et al.*, 2009).

A number of plants in Africa are also used for the management of livestock diseases. For instance, *Commiphora holtziana* has been utilized by rural pastoralist communities for repelling ticks and other harmful insects. This species is widely distributed in Kenya, Uganda,

Tanzania, Ethiopia, and Somalia. It secretes gum resin called 'gum haggar' which is rich in sesquiterpenes and furanosesquiterpenes (Birkett *et al*., 2008). The resin from this plant was reported to exhibit repellency activity against cattle ticks (*Boophilus microplus*) and red poultry mite (*D. gallinae*) in chickens. The repellency was later established to be due to sesquiterpenes identified as gamma-elemene (16.69%), beta-bourbonene (20.83%) and germacrene-D (11.64%) (Birkett *et al.*, 2008). Most of the species in the genus *Commiphora* have also been reported to exhibit acaricidal and insecticidal activities. Other plant species exploited for the management of livestock diseases include *Maesa lanceolata*, *Myrsine africana*, *Rapaneamelan ophloeos*, *Embelia schimperi* and *Embelia keniensis*. They are used by many ethnic groups in Kenya for the treatment of intestinal worm in animals. Midiwo *et al*. (2003) validated this use and went further to establish the presence of benzoquinoid that reportedly exhibited nematicidal activity.



Compounds reported from *Tagetes minuta*

Discussion

This review aimed at documenting the plants and their bioactive compounds responsible for treatment of veterinary diseases. The insecticidal and anthelmintic activity of these plants may be attributed to the presence of bioactive compounds in different morphological parts of the plants. The results of some research findings showed that the repellence activity of some plants was due to presence of essential oils but the composition varies according to the different parts of the plants and its stage of growth (Taylor *et al.*, 2011). Apart from insecticidal and anthelmintic activity of these plants, there are other research findings which showed that these plants can be used to treat different diseases in humans. The study done by Uzabakiriho *et al*. (2015) showed that *T. minuta* methanolic and water extract had high antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* which cause disease to human.

Acknowledgement

I wish to acknowledge the RUFORUM through RUFORUM CAA Project (RU 2015 GRG – 127) project for funding my studies at Nelson Mandela African Institution of Science and Technology. This paper is a contribution to the 2016 Fifth African Higher Education Week and RUFORUM Biennial Conference.

References

Bachaya, H.A., Iqbal, Z., Khan, M.N., Jabbar, A., Gilani, A.H. and Islam-Ud-Din. 2009. *In vitro* and *In vivo* anthelmintic activity of Terminalia arjuna bark. *International Journal of Agriculture and Biology* 11(3):273–278.

Birkett, M.A., Abassi, S.A., Kröber, T., Chamberlain, K., Hooper, A.M., Guerin, P.M. and Wadhams, L.J. 2008. Antiectoparasitic activity of the gum resin, gum haggar, from the East African plant, Commiphora holtziana. *Phytochemistry* 69(8):1710–1715.

Dut Jasuja, N., Choudhary, J., Sharama, P., Sharma, N. and Joshi, S. 2012. A review on bioactive compounds and medicinal uses of *Commiphora mukul*. *Journal of Plant Sciences* 7(4):113–137.

Hulina, N. 2008. Wild marigold - *Tagetes minuta* L ., New weed on the Island of Hvar , and new contribution to the knowledge of its distribution in *Dalmatia* (Croatia). *Agriculturae Conspectus Scientificus* 73(1):23–26.

Makkar, H.P.S., Norvsambuu, T., Lkhagvatseren, S. and Becker, K. 2009. Plant secondary metabolites in some medicinal plants of Mongolia used for enhancing animal health and production. *Veterinary Research*. pp. 159–167.

Midiwo, J.O., Yenesew, A., Juma, B.F., Derese, S., Ayoo, J.A., Aluoch, A.O. and Guchu, S. 2003. Bioactive compounds from some Kenyan ethnomedicinal plants/ : *Myrsinaceae, Polygonaceae* and *Psiadia punctulata.* pp. 311-323.

Ofori, D., Anjarwalla, P., Mwaura, L., Jamnadass, R., Stevenson, P. and Smith, P. 2013. Pesticidal plant leaflet. pp. 6–7.

Taylor, P., Moghaddam, M., Omidbiagi, R. and Sefidkon, F. 2011. Chemical composition of the essential oil of *Tagetes* chemical composition of the essential oil of *Tagetes minuta* L . (November 2014), 3–5.

Uzabakiriho, A., Sinayobye, O.J. and Habimana, S. 2015. Antibacterial effects of *Tagetes minuta* plant extracts against E*scherichia coli* and *Staphylococcus aureus* in Rwanda 2(2):44–48.