EFFICACY OF ALLIUM SATIVUM, ALLIUM CEPA AND JATROPHA CURCAS ON COMMON NATURAL GASTROINTESTINAL HELMINTHS IN DOGS

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DECLARATION

This research is my original work and has not been presented for award of a degree in any other university. I have also given appropriate credits to the citations of the work of other authors.

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DEDICATION

I would like to dedicate this work to my daughters Najma and Hanifah since they have given me a renewed sense of purpose to pursue these studies and to my wife Habon for her unlimited support and encouragement. I also wish to dedicate this work to my parents, my sister and all my brothers for bringing me up wonderfully.

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ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
ASAL	Arid and Semi-Arid Lands
EPG	Eggs Per Gram
FAO	Food and Agriculture Organization
GIT	Gastro Intestinal Tract
HCT	Hematocrit
HGB	Hemoglobin
HIV	Human Immunodeficiency Virus
LARMAT	Land Resource Management and Agricultural Technology
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
OECD	Organization of Economic Co-operation and Development
RBC	Red Blood Cells
SPSS	Statistical Package for Social Sciences
USDA	United States Department of Agriculture
WBC	White Blood Cells

ABSTRACT

Prevalence of animal diseases is one of the major livestock production constraints in Kenya with high impacts on livelihoods due to related economic losses affecting food security in the country. The use of synthetic drugs for disease management has challenges; this makes the use of medicinal plants for treatment a rational alternative. Resistance to drugs that are used for the treatment of infections caused by parasitic helminths is an inevitable consequence that could be attributed to excessive treatment frequency, under-dosing and the use of long-acting formulations that decline in activity over time. Helminths of zoonotic importance in dogs in Kenya include *Toxocara canis*, *Ancylostoma caninum* and *Dipylidium caninum* which are commonly found in intestines of dogs and can cause infestation in human beings. The purpose of this study was to investigate the *In vitro* and *in-vivo* efficacy of aqueous and ethanol extracts from bulbs of *Allium cepa* and *Allium sativum* as well as leaves of *Jatropha curcas* against common gastrointestinal helminths of dogs. Six extracts from the three plants were selected for *in vitro* anthelmintic screening by measuring ability to inhibit egg hatching, egg development and survival of larvae. For *in vivo* efficacy, fifteen puppies of mixed sexes, aged between 8 and 10 weeks, with an average weight of 2.2 kg were grouped into three with five animals in each group; Group 1 was treated with the extract, group 2 was given the recommended dose of a commercial anthelmintic while group 3 was given distilled water, all as single treatments. Fecal samples were obtained from each puppy a day before treatment (day 0) and on days 1, 3, 5, 7, 10 and 14 post treatment for determination of Eggs Per Gram (EPG). The anthelmintic effects were established by computing the percentage reduction of eggs shed in feces (fecal egg count reduction %FECR) using the pretreatment and post treatment EPG counts. Whole blood was collected from each puppy on days 0, 7 and 14 to determine changes in the hematological parameters. Two puppies from each group were then randomly selected and sacrificed for postmortem examination and for collection of intestinal contents for total worm counts and identification.

The ethanol extracts of *A. cepa* inhibited hatching of 100% of eggs of *A. caninum* between 10,000 ug/ml and 2,500 ug/ml and 100% of eggs of *T. canis* between 10,000 ug/ml and 1,250 ug/ml while that of *A. sativum* inhibited hatching of 100% of *A. caninum* eggs between 10,000 ug/ml and 5,000 ug/ml. However the ethanol extract of *A. sativum* did not have similar effect on the development of *T. canis* eggs at these concentrations. The ethanol extracts of both *A. cepa* and *A. sativum* affected the survival of 100% of *A. caninum* larvae at a concentration of 156 ug/ml and higher. The aqueous extracts of the three plants had moderate effects on the eggs and the larvae of both parasites. Ethanol extract of *A. cepa* was found to be the most effective *in vitro* and therefore was tested for *in vivo* efficacy. When tested *in vivo* at a dose of 6 mg/kg of body weight, the ethanol extract of *A. cepa* produced a percentage fecal egg count reduction of 47% for strongyle eggs. A significant drop in WBC ($p=0.035$) was observed 7 days after treatment and a significant increase in RBC ($p=0.04$) and HGB ($p=0.001$) 14 days after treatment. The changes in hematological parameters when compared among the control and treatment groups were found to be significant ($p<0.05$) 7 days after treatment for WBC, RBC, HGB and HCT, and 14 days after treatment for MCHC. No toxicity signs were observed following oral treatment with the ethanol of *A. cepa* extract at 6 mg/kg. On post mortem examination, the sacrificed animals were anemic with foci of congestion and hemorrhages on intestinal mucosa. Adult ascarids, hookworms and whip worms were isolated from their intestinal content although fewer in animals from the positive control group. The results indicate that the ethanol extracts of *A. cepa* and *A. sativum* have anthelmintic properties which can be investigated further to support the ethnoveterinary use of the plants as anthelmintic drugs for control and treatment of worm infestation in dogs. The 47% efficacy against hookworms observed in treated puppies was due to the anthelmintic properties of the crude ethanol extract of *A. cepa*. This was supported by the hematological changes which occurred as a result of administration of the extract.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Prevalence of Animal diseases is one of the major livestock production constraints in Kenya (Onono *et al.* 2013) with high impacts on livelihoods due to related economic losses. These diseases also affect food security in Kenya. The use of synthetic drugs for disease management has always been a challenge because of the unavailability of these drugs especially in rural areas, shortage of foreign exchange to import them, lack of finance to purchase them, drug resistance, misuse due to paucity of knowledge and environmental pollution (Gakuya *et al.* 2011), making the use of medicinal plants a rational alternative.

One of the branches of ethnobiology is ethnobotany, which describes how the use and management of plants are perceived by human beings including uses as medicine (Choudhary and Pillai, 2008). Ethnobotanical surveys carried out in many parts of Kenya have revealed that many plants are being used in animal disease management. The beliefs, practices, knowledge, skills or methods applied in the use of medicinal plants for treatment of animals is referred to as ethnoveterinary medicine (Evelyn *et al.* 2001; McCorkle, 1986). For continuation of traditional approaches to animal health, ethnoveterinary knowledge should be regularly updated especially in poor countries where local communities depend on livestock production for livelihoods (Abbasi *et. al.* 2013).

Plant secondary metabolites are known to have antiparasitic properties, depending on their structure, levels of ingestion and bio availability (Athanasiadou and Kyriazakis 2004). For example plants that contain condensed tannins have been found to have antioxidant, antifungal and antimicrobial activity (Suraya *et al.* 2011: Andréia *et al.* 2005).

Parasitic diseases are the most common diseases in animals. Organisms causing these diseases can be broadly categorized as bacteria, fungi, viruses, protozoa and helminths. Helminthoses refers to a complex of conditions caused by helminth parasites of the classes' Nematoda, Cestoda and

Trematoda (Githiori *et al.* 2004). The definitive classification of helminthes as described by Gilbert (1996), is done by observing the distinct features of eggs, larvae and adult worms. Adult trematodes are leaf like with prominent suckers for maintaining position. Adult tapeworms (Cestodes) are elongated, segmented and hermaphroditic flat worms inhabiting the lumen of the intestines. Their larvae may be solid or cystic in consistency and inhabit tissues outside the gastrointestinal system. Adults and larvae of roundworms (Nematodes) are bisexual, cylinder shaped and inhabit intestinal and non-intestinal tissues. In dogs and cats; hookworms, whipworms, ascarids, coccidia, tapeworms, and heartworms are frequently observed (Kagira and Kanyari 2000). *Toxocara canis*, *Ancylostoma caninum* and *Dipylidium caninum* are helminths commonly found in the intestines of dogs and can cause helminthiases in human hence are of zoonotic importance.

Treatment of infections caused by parasitic helminths in animals is by the use of anthelmintic drugs. Most modern anthelmintics are prepared as combination drugs for treatment of mixed gastrointestinal helminth infestations. In Kenya, the most commonly used drugs for the treatment and control of helminth infestations in dogs are a combination of Febantel, Pyrantel Permoate and Praziquantel such as Vermic Total^{TM}.

Resistance to anthelmintic drugs was first reported in 1957 on phenothiazine (Drudge *et al.* 1957). Key operational factors which have been identified as promoting resistance include excessive treatment frequency, under-dosing and the use of long-acting formulations that decline in concentration over time (Hughes *et al.* 2007 Jabbar *et al.* 2006 Besier and Love, 2003 Waller, 1986 Prichard *et al.* 1980). These have necessitated the need for a continuous search for new anthelmintic agents.

Studies have been designed to determine efficacy of a compound and to examine whether treatment with the compound will lead to the desired or harmful outcomes in controlled conditions (Singal *et al.* 2014). Acute toxicity studies are carried out in order to get information about the mechanisms of

action of a chemical in the body as well as information on the risks associated with handling of chemicals and how to manage these risks (Walum, 1998).

1.2 Problem statement

Poverty in rural areas of Kenyan is a widespread phenomenon (Ayako *et al.* 1997) and a majority of people living in these areas keep animals as a source of their livelihood. Many of the households keep dogs which host parasites of zoonotic importance including *Toxocara canis, Ancylostoma caninum* and *Dipylidium caninum*. High costs associated with use of synthetic drugs and access to Veterinary services make the treatment of parasitic infections using medicinal plants a viable option for people living in the country side. With the significance of keeping animals in the Kenyan economy, a lot of work needs to be done in order to develop knowledge on alternative medicines with the aim of increasing profitability of these poor people's animal enterprises by reducing production costs. Furthermore, there is a need to develop new anthelmintic drugs because of development of resistance against available drugs.

1.3 Purpose of the study

The study focused on anthelmintic activity of ethanol and aqueous extracts of *Allium cepa, Allium sativum* and *Jatropha curcas* against common gastrointestinal helminths of dogs. Anthelmintic activity was tested *in vitro* for all the extracts and *in vivo* in puppies, 8-10 weeks of age, for the ethanol extract of *A. cepa* which was found to be the most effective extract *in vitro*. Acute toxicity studies were carried out on the ethanol extract of *A. cepa* in order to determine the margins of safety using the OECD guidelines of 2001 for testing of chemicals and applying the test procedure with a starting dose of 300 mg/kg body weight.

1.4 Objectives of the study

The general objective of the study was to determine the anthelmintic efficacy and safety of extracts of *Allium sativum, Allium cepa* and *Jatropha curcas.*

Specific objectives of the study were to;

1. Establish the *in vitro* anthelmintic effects of the ethanol and aqueous extracts of *Allium sativum, Allium cepa* and *Jatropha curcas* on worm eggs and larvae.
2. Establish the *in vivo* anthelmintic effects of the ethanol extract of *Allium cepa* in puppies.
3. Test the acute toxicity of the ethanol extract of *Allium cepa* in rats.

1.5 Significance of the study

Due to the development of resistance to modern anthelmintic drugs, it is necessary to search for new anthelmintic drugs which can combat resistant helminthes thereby reducing the prevalence of diseases caused by these helminths and improve livestock productivity. It has been reported in studies done by other researchers that various plant extracts have activity against different species of helminthes. Information from this study will be useful to researchers in drug development and will add to the body of knowledge and the use of medicinal plants in the treatment of diseases caused by helminthes in livestock.

1.6 Hypotheses

The two hypotheses for the study were;

1. The aqueous and ethanol extracts of *Allium sativum, Allium cepa* and *Jatropha curcas* have both *in vitro* and *in vivo* anthelmintic effects on common gastrointestinal helminths of dogs.
2. The aqueous and ethanol extracts of *Allium sativum, Allium cepa* and *Jatropha curcas* have no signs of acute toxicity when administered orally in dogs at the selected dose.

CHAPTER TWO

LITERATURE REVIEW

2.1 Medicinal plants

These are plants used in herbal medicine and are known to have chemical constituents that are used in development of drugs, some of which have been used for treatment of parasitic conditions. These chemical constituents have been used to develop laxatives, anticoagulants, antibiotics, antimalarial drugs among others. Specific examples include morphine from opium poppy, vincristine from periwinkle and taxol from foxglove among others (Rasool and Bassam 2012). These ingredients are broadly referred to as phytochemicals.

2.2 Plants with anthelmintic activity in animals

Selected plants which have been reported to have efficacy against helminths of Veterinary importance include; Acacia species *(Acacia auriculaeformis, Acacia oxyphylla* and *Acacia caesia*), *Artemisia absinthium, Artemisia vulgaris, Azadirachta indica, Bursera copallifera, Butea monosperma, Caesalpinia crista, Calotropis procera, Carica papaya, Centratherum (Vernonia) anthelminticum, Chenopodium album, Commiphora mukul, Dryopteris filix-mas, Gynandropsis (Cleome) gynandra, Hagenia abyssinica***,** *Juniperus communis, Manihot esculenta***,** *Mimusops elengi, Nicotiana tabacum, Neolamarckia cadamba, Phytolacca icosandra***,** *Piper longum, Sarothamnus scoparius, Sericea lespedeza***,** *Trifolium repens, Xylopia aethiopica* among others**.** (www.parasitipedia.net)

2.3 Extraction of Plant Materials

Extraction is the crucial initial step in the process of analysis of medicinal plants. The process removes a wanted chemical constituent of a plant for purification and identification and involves washing, drying, grinding, dissolution, filtration and concentration (Cos *et al.* 2006). Drying plant materials before extraction reduces the risks of degradation, biochemical changes, contamination by microorganisms and also facilitates accuracy in sampling because dry tissues are easy to grind (Celso

and Ladaslav 1995). Air drying and oven drying are commonly used as drying methods. Grinding is done to homogenize the sample, facilitate kinetics analytic extraction and increase the surface area of the sample in contact with the solvent (Cos *et al.* 2006).

The solvent used for extraction is selected based on the solubility of the targeted compound. Ethanol extraction yields higher concentrations of hydrophobic components of the plants while aqueous extraction will retain higher concentrations of the hydrophilic components (Navrozedeep, 2010). Solvent removal is an essential process following dissolution and filtration of the plant materials. Water extracts are best dried by lyophilization. This is because biochemical properties of the components dissolved in water are conserved and stability of these components is increased. However the process has disadvantages in the costs, increased handling and processing time (http://www.pharmacistspharmajournal.org). Ethanol extracts are dried by evaporating the solvent in an open container on a steam/sand bath or by using a rotary evaporator.

Dry crude extracts are reconstituted to the required test concentrations using Dimethyl Sulfoxide (DMSO) (Adamu *et al.* 2013) water or the respective extracting solvents (Abubakar, 2010), before making serial dilutions. Dimethyl Sulfoxide or Tween are suitable for reconstituting dry extract which are not soluble in water. However, some concentrations of DMSO have been found to have antimicrobial activity (Muhamad, 2006). Worms can tolerate up to 2% of DMSO or Tween80, although at this percentage extracts made with pure ethanol will not dissolve (Luciana *et al.* 2011).

2.4 *Allium sativum*

Allium sativum is a bulbous monocotyledonous plant that lives for more than two years. Relatives include *A. cepa* (onions and shallots) and *A. ampeloprasum* (leeks). “Sativum” means “cultivated”. It is not found in the wild but appears to have originated in mountainous regions in Central Asia (Brewster, 2008). For centuries garlic has been used for medicinal purposes and investigations for its medicinal value have gone on over several years (Onyeagba *et al.* 2004)

According to Friesen *et al*. (2006), *A. sativum* is classified as belonging to the class Liliopsida, subclass Liliidae, Superorder Liliianae, order Amaryllidales, family Alliaceae, subfamily Allioideae, tribe Allieae and genus Allium. Sulphur compounds in *A. sativum* are responsible for its characteristic smell and medicinal properties. The concentration of these compounds is high in *A. sativum* when compared to other allium species. Allicin (diallyl thiosulfinate or diallyldisulfide) has been reported to be the most active compound in *A. sativum* while alliin (S-allylcysteine sulfoxide) is the most abundant (Gebreselema and Mebrahtu 2013).

It contains 17 amino acids, vitamins, minerals and 33 sulphur compounds (Peter *et al.* 2008). Constituents of Allium sativum extracts found to have anti-parasitic effects include; ajoene (Gebreselema and Mebrahtu 2013; Ledezma and Apitz-Castro 2006), allicin (Josling, 2001), allyl methyl thiosulfanate and ethyl allyl thiosulfanate (Gebreselema and Mebrahtu 2013), diallylmonosulfide, diallyldisulfide, and diallyltrisulfide (Tsao and Yin, 2001)

2.4.1 Past studies on *Allium sativum*

Studies have been conducted to demonstrate the medicinal value of the garlic. This review will focus on the activity against common parasites of Veterinary importance.

Studies by Josling, (2001) showed that prophylactic treatment with a supplement containing allicin prevented infection by the virus that causes common cold. Ajoene, allyl methyl thiosulfanate and ethyl allyl thiosulfanate found in *A. sativum* have been reported to kill viruses (Gebreselema and Mebrahtu 2013).

Reports by Johnston, (2002) indicated that allicin is mainly responsible for the antibacterial effects of garlic. Volatile compounds present in garlic such as diallylmonosulfide, diallyldisulfide, and diallyltrisulfide have antimicrobial properties against both gram positive and gram negative bacteria. (Tsao and Yin 2001).

Results from studies conducted by Shams-Ghahfarokhi *et al.* (2006) show that there was activity of garlic against *Malassezia furfur, Candida albicans,* other candida species and dermatophytes. The garlic extract ajoene is responsible for the antifungal activity (Ledezma and Apitz-Castro 2006).

In vitro studies have shown anthelmintic activity of garlic against *Fasciola gigantica* (Kumar, 2014), *Schistososma mansoni* (Mantawy *et al*. 2012) and *Hemonchus contortus* (Zafar, 2014).

A study performed to investigate the activity of *A. sativum* against *Aspiculuris tetraptera* using naturally infected Swiss albino mice, freshly cut garlic was found to be effective against the parasite (Ayaz *et al.* 2008). However, in a study conducted to evaluate the effectiveness of garlic in controlling gastrointestinal parasites in adult female boar goats naturally infected with *Haemonchus contortus,* anthelmintic effects were not observed (Worku *et al.* 2009). *A sativum* did not reduce the count of strongyle eggs shed in feces following treatment of donkeys using extracts from the plant (Sutton and Haik 1999).

In studies of activity of *Allium sativum* on ectoparasites, dichloromethane extract of *Allium sativun* bulbs exhibited activity on *Hyalomma marginatum rufipes* and *Rhipicephalus pulchellus* ticks (Nchu *et al*. 2005). Extracts of the plant have exhibited activity on *Boophilu annulatus* (Shawky 2013) and *Damalinia caprae* (Bindu *et al.* 2013).

In studies of activity of *Allium sativum* against protozoan parasites, Sabah Ahmed, (2010) observed in vitro activity of *Allium sativum* against *Trichomonas vaginalis*. Extracts of *Allium sativum* combined with diminazene aceturate were found to have combined effects on parasitemia of *Trypanosoma brucei* in experimentally infected rats (Peni *et al*. 2012).

Acute toxicity studies of garlic extract were carried out in Wistar rats and ddY mice with no specific signs observed after use of garlic extract in survivals for 7 days (Nakagawa *et al.* 1984). A similar study done in male Sprague dowly rats established that garlic extracts did not produce any visible change in the histopathological examinations of the kidneys, heart, lungs and prostrate (Isaac *et al.* 2014).

Acute toxicity study of *A. sativum* in rabbits using different dose levels given through the subcutaneous route, LD_{50} was found to be 3034 mg/kg BW and 2200 mg/kg BW as the maximum tolerated dose. Toxic signs observed at doses between 3200 mg/kg BW and 4200 mg/kg BW include inappetance, partial paralysis and death (Mikail, 2010).

Toxicity studies carried out to investigate whether *A. sativum* can cause allergy; diallyl disulfide, allylpropyl sulfide and allicin found in the plant were reported to cause allergy (Barnes *et al.* 2007; Papageorgiou, 1983). Findings from other toxicology studies of *A. sativum* reported that extracts of the plant caused suppression in hemoglobin synthesis and destruction of red blood cells leading to anemia in experimental animals (Barnes *et al.* 2007).

2.5 *Allium cepa*

Allium cepa is a bulbous plant with a single cotyledon, harvested twice a year and is the most cultivated compared to other allium species (www.eol.org). Sulphur compounds in *A. cepa* are responsible for its characteristic smell, taste, eye irritation and medicinal properties (Brewster, 1994). *Allium cepa* is classified in the class Liliopsida, subclass Liliidae, order Liliales, family Liliaceae and genus *Allium* (USDA)

Phytochemical screening of Allium cepa showed that it contains alkaloids, flavonoids, cardiac glycosides, tarpenes and resins (Gazuwa *et al*. 2013). *Allium cepa* contains quercetin and thiosulphonate which are responsible for medicinal benefits (Kumar *et al*. 2010).

2.5.1 Past studies on *Allium cepa*

Studies have been conducted in order to demonstrate the medicinal value of the onion. This review will focus on the activity against parasites of veterinary importance.

Flavonoids in *Allium cepa* have been found to have activity against the Human Immunodeficiency Virus (HIV), herpes simplex, Influenza and Rhinovirus (Tsanova-Sanova, 2011)

Allium cepa caused up to 100% dose dependent inhibition on the growth of *Malassezia furfur*, candida species and other dermatophytes (Shams-Ghahfarokhi *et al.* 2006). Growths of gram

negative and gram positive bacteria were also inhibited. Fresh onion extract was observed to inhibit the growth of the bacteria *Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes,* and *Streptococcus pneumonia* (Shinkafi and Dauda, 2013). Inhibitory effects on *Vibrio cholera* have also been demonstrated (Hannan *et al.* 2010).

An *in vitro* study of crude extract of *Allium cepa* bulbs revealed strong anthelmintic activity on *Pheretima posthuma* (earthworm) as both the ethanol and aqueous extracts of the plant caused paralysis within 20 minutes and death within 30 minutes of exposure (Abhijeet *et al*. 2012). *A. cepa* combined with *Cocos nucifera* taken in food stopped gastrointestinal infections in sheep (Heinz *et al.* 2010). The combination has been found to have marked activity against adult *Trichuris muris* and adult *Hymenolepis microstoma* in mice (Abu, 2005).

Allium cepa was found to have activity against *Boophilus annulatus* (Shawky, 2013) and *Trichomonas vaginalis* (Sabah, 2010).

A coloring agent of food extracted from *A. cepa* and fed to mice had no acute and sub-acute toxic effects in both sexes (Kojima *et al.* 1993). However, consumption of *Allium cepa* by cattle, cats, dogs, sheep and goats may cause toxicity due to anemia and impaired oxygen transport (Cope, 2011). Toxicity studies of the effects of ethanol extract on fertility in rats have been reported, the extract inhibited implantation in a statistically significant manner but had no effect on ovulation in female rats pretreated with a dose of 300 mg/kg of body weight (Vishnu *et al.* 2009).

2.6 *Jatropha curcas*

Jatropha curcas is a monoecious plant with unisexual or occasionally hermaphrodite flowers which have 3 long slender stalks which are cone shaped each with a large stigma divided into two. It grows to a height of 3-5 m.

The branches have a thick milky sap. The number of roots formed vary depending on whether the plant is propagated from seeds of vegetative parts.

Leaves have 5-7 lobes. It has inflorescence each yielding more than 10 oval shaped fruits. The seeds are black and weigh approximately 0.73 g per seed (Kumar and Sharma 2008). The plants is well distributed in Kenya and is increasingly being known due to its documented potential for bio fuel production of some of its species.

2.6.1 Past studies on *Jatropha curcas*

A number of studies have been conducted to demonstrate the medicinal value of *Jatropha curcas*. This study will review its activity against parasites of veterinary importance.

In vitro studies of chloroform and hexane extracts from seeds of *J. curcas* have reported inhibition of growth of *S. aureus, K. pneumoniae, P. aeuriginosa, S. typhimurium* as well as candia, aspergillus, rhizopus and mucor species of fungi (Sundari and Selvaraj 2011). Similar activity was noted with the stem bark extracts (Igbinosa *et al*. 2009), root bark extracts (Namuli *et al*. 2011) and leaf extracts (Kalimuthu *et al.* 2010) of the plant.

Antifungal activity has been reported with the ethanol extracts from leaves of *Jatropha curcas* against common dermatophytes; Trichophyton, Microsporum, Epidermophyton together with *Malassezia furfur* (Mbakwem *et al.* 2012).

Hexane, ethyl acetate and ethanol extracts obtained from the seeds of *Jatropha curcas* were found to have *in vitro* activity against *Haemonchus contortus* (Monterio *et al.* 2011). Whereas the aqueous latex of leaves of *Jatropha curcas* has been reported to have *in vitro* activity against *Pheretima posthuma* (Hitesh *et al*. 2014). Leaf extracts of *Jatropha curcas* fed with meals to naturally infected chicken was reported to reduce helminthosis caused by *Ascaridia galli* (Suharti *et al.* 2010). Extracts from leaves of *Jatropha curcas* were reported to have effective anti-viral activity by inhibit the entry of drug resistant Human Immunodeficiency Virus (Dahake *et al.* 2012).

Activity of *Jatropha curcas* against common ectoparasites has been investigated. Studies in rabbits done by addition of *J curcas* seed meal in food at a ratio of 1:9 and feeding to rabbits was found useful in control of ectoparasites (Abdel-Shafy *et al.* 2011). Treatment of *Boophilus annulatus* with

ethanol extracts from leaves of *J. curcas* inhibited hatching of laid ova (Sanis *et al.* 2012). Whereas, studies by Mawela, (2008) reported that methanol extracts of *Jatropha curcas* repelled *Rhipicephalus appendiculatus* to a statistically significant level. Hexane, ethyl acetate and methanol extracts of *Jatropha curcas* linn also displayed remarkable anti-trypanosomal activity (Abiodun *et al.* 2012).

Toxicity studies of *Jatropha curcas* have been done in mice (Abdu-Aguye *et al.* 1986) using sodium chloride seed extracts) and (Cai Yan *et al.* 2010) using phorbol esters as well as in calves (Ahmed and Adam, 1979) using seeds.

2.7 Parasitic helminths in dogs

About 14% of deaths in dogs in Kenya are caused by parasitic conditions, 68% of these deaths are attributed to gastrointestinal helminth infestations of which 41% are caused by *Ancylostoma caninum*. Other helminth parasites identified to be causing death in dogs are *Toxocara canis* and *Dipylidium caninum*. Puppies and adult dogs older than 8 years were mostly affected (Kagira and Kanyari 2000). Prevalence studies of gastrointestinal parasites in dogs have been done in many parts of the world but none in Kenya. In a similar study in Durban and Coast of South Africa the following parasites and their prevalence were detected; Ancylostoma sp. (53.8 %), *Trichuris vulpis* (7.9 %), *Spirocerca lupi* (5.4 %), *Toxocara canis* (7.9 %), and *Toxascaris leonina* (0.4 %). Others helmiths of importance in dogs are *Dipylidium caninum, Echinococcus granulosa*, *Taenia ovis, Taenia hydatigena* and *Dirofilaria immitis*. Dogs harbouring a single parasite species were more common (41.7 %) than those harbouring two (15 %) or multiple (2.1 %) species (Mukaratirwa and Singh 2010). Amongst these helminths of dogs, tapeworms are; *D. caninum, E. granulosa, T. ovis* and *T. hydatigena. A. caninum* is the hookworm, while roundworms are *T. canis, T leonina, S. lupi*, and *D. immitis*. Those of zoonotic importance are *D. caninum, E. granulosa, T. ovis, T. hydatigena, T. canis, D. immitis* and *A. caninum.* This review will focus on *T canis* and *A. caninum*.

2.8 Development of Anthelmintic Resistance

Resistance to anthelmintic drugs develops when worms that were sensitive to a particular drug loose sensitivity through genetic mutation. The mutation may be triggered by excessive treatment frequency, use of sub-therapeutic doses and the use of long-acting formulations that decline in concentration over time and creates alternative forms of genes that are responsible for resistance (Kohler, 2001). These genes that code for resistance will be present in subsequent generations of worms even without exposure to the drugs corresponding to the resistance genes therefore making the worms less sensitive. The higher the number of worms carrying the resistance genes in a worm population, the higher the number of worms that will survive exposure to corresponding drugs and therefore the higher the rate of development of anthelmintic resistance in that worm population (Carl, 2010).

2.9 *Toxocara canis*

The parasite has a worldwide distribution prevalent in all locations that have domestic dogs and other canids. It inhabits the gastrointestinal tract of the definitive hosts and infection is by ingestion of eggs. The worm is dioecious having morphology distinctly different between the sexes. Male are 4-6 cm long and are smaller than females which are 6.5-15 cm. both males and females have three prominent lips each with a dentigerous ridge. The lateral hypodermic cords are visible with the naked eye. There is no gubernaculum. The rear end of the male has a ventral curve and a blunt pointed tail. The eggs are brownish almost spherical measuring 75-90 um (Harris-Linton, 2001).

2.9.1 Life Cycle of *Toxocara canis*

Life cycle of *Toxocara canis* as described by Colin, 2000; Eggs of *T. canis* develop to infective stages in 2-6 weeks (Azam *et al*. 2012). Dogs and other canids are definitive hosts in the life cycle of *T. canis* where the adult worms are commonly found in the intestines. Human beings and rodents are infected as intermediary hosts where the infective larval stages persist in somatic tissues without

further development. Dogs older than 6 months are infected directly by ingesting infective eggs or indirectly by ingesting infected mice while nursing puppies are infected by ingesting infective larval stages (L3) through their mother's milk. Unborn puppies are infected by migrating infective larval stages (L2) from their mothers tissues across the placenta into the fetal liver where they remain until birth after which the resume migration to the lungs of the newborns. From the lungs they migrate through the trachea to the pharynx from where they are swallowed into the intestines where they develop to adult worms. Following ingestion of infective larvae (L3) either directly or indirectly from the intermediary hosts, a proportion of the larvae undergo migration through the lungs to the heart from where they are distributed to the body tissues hence the presence of infective stages in milk and fetal liver (Colin, 2000).

2.10 *Ancylostoma caninum*

It is a nematode species known as the hookworm, which principally infects the small intestine of dogs and other canids (Sofia, 2003). Females are larger than males typically 14–16 mm long and 0.5 mm wide, while the males are smaller at 10–12 mm in length and 0.36 mm in width (Marquardt and Grieve, 2000). Eggs are laid by the females typically when at the 8-cell stage, the eggs are 38-43 μm in width with thin walls (Olsen, 1986).

2.10.1 Life Cycle of *Ancylostoma caninum*

Dogs and other canids are definitive hosts in the life cycle of *A. caninum*, in which adult worms are commonly found in the intestines. Eggs are shed in feces and hatch within 24-48hrs in favorable conditions and develop to infective larvae (www.cdc.gov/dpdx/hookworm). The infective stage of the parasite is the larval stage L3. Dogs get infected when the larvae (L3) attach to the skin and enter the circulatory system through the hair follicles. From the circulatory system, a portion reaches the pulmonary circulation into the lungs and through the trachea, eventually getting to the upper respiratory tract and are swallowed into the gut where they develop to L4 which attaches to the

intestinal walls and develop further to adult stage. This pathway is common in puppies. The other proportion is deposited into muscles and do not continue developing. This pathway is common in adult dogs. Infection may also occur through direct ingestion of L3 into the gut where development to L4 takes place. Nursing puppies are infected through their mother's milk following activation of somatic L3 stages during gestation and subsequent migration to mammary tissues. In cases of trans mammary infection, puppies begin shedding eggs in feces 14 days after birth (Alex and Paul 2001).

2.11 Treatment of parasitic helminths in dogs

In Kenya, the most commonly used drugs for the treatment and control of helminth infestations in dogs are a combination of Febantel, Pyrantel Permoate and Praziquantel such as Vermic Total™. Amongst the most prevalent helminths of dogs, Febantel acts on *A. caninum, D. caninum, T. canis* and *T. vulpis*, Pyrantel Permoate acts on *A.caninum, T. canis and T. leonina* while Praziquantel acts on *D. caninum, E. granulosus, T hydatigena* and *T. ovis*. Ivermectin is commonly used in prevention of *D. immitis*. Sometimes helminth infestations shows symptoms of severe allergy which are treated using corticosteroids (Dickson, 2003).

Benzimidazoles have a biochemical mode of action, they bind selectively to beta-tubulin and inhibit microtubule formation while pyrantel is an agonists at nicotinic acetylcholine receptor sites of nematode muscles causing spastic paralysis (Martin, 1997)

2.12 *In Vitro* and *In Vivo* Assays

The major methods for detection of anthelmintic resistance and efficacy is the fecal egg count reduction tests (FECRT) in vivo and the egg hatch test (EHT) in vitro, both of which can be used with all anthelmintic groups (Coles *et al*. 2006).

The egg hatch assay (EHA) is an in vitro method standardized at laboratory level for detection of benzimidazole resistance in nematode parasites of small ruminants (Calvete *et al.* 2014), and can also be used for efficacy studies of other potential anthelmintic drugs. Larval Survival Assays (Larval

Mortality Assays) can be done after determination of EHA by further incubation and calculating the percent death comparing the live and dead worms (Thiothi *et al.* 2002).

Since sensitivity to test drugs decreases with the age of the eggs, in *in vitro* studies eggs should be used within 3 hrs of collection (Coles *et al.,* 2006). *T. canis* eggs have a resistant outer shell which give them the ability to withstand many disinfectants and remain viable for several years in moist, shaded soils when temperatures are cool (Harris-Linton, 2001).

2.13 Toxicology of *A. cepa, A. sativum* and *J. curcas*

The ratio of the highest exposure to a chemical that results in no toxicity compared to the exposure which produces the desired efficacy is referred to as the therapeutic index (Patrick and Mark 2012). Therapeutic index is an important parameter in achieving a balanced safety-efficacy profile for a given indication.

A summary of acute toxicity of the three medicinal plants is given below.

Studies of acute toxicity of *A. cepa* in rodents established the LD_{50} to be 3000 mg/Kg body weight in rodents (Shenoy *et al.* 2009). From acute toxicity study of *A. sativum* in rabbits using different dose levels given through the subcutaneous route, LD_{50} was found to be 3034 mg/kg BW and 2200 mg/kg BW as the maximum tolerated dose. Toxic signs observed at doses between 3200 mg/kg BW and 4200 mg/kg BW include inappetance, partial paralysis and death (Mikail, 2010). While the LD_{50} of phorbol esters extract of *J. curcas* in mice were 27.34 mg/kg body mass (Cai-Yan Li, 2010).

2.14 Hematology associated with helminthiasis and extracts from the selected medicinal plants

Hematological and biochemical changes occur following worm infestations, using hematological parameters to evaluate toxicity of a compound is pertinent because changes in these parameters have a high probability of being associated with toxic effects of the test compound compared to changes in other body systems (Nwaka *et al.,* 2015). A study conducted in dogs infected by *Dirofilaria immitis* found the main hematological changes to be anemia in 38.71%, increased numbers of White Blood

Cells in 29 %, peripheral eosinophilia in 38.71 %, basophilia in 35.48 %, and decreased hemoglobin (HgB) concentration in 19.35% of the examined dogs (Lefkaditis *et al.* 2009)

A dose dependent and statistically insignificant drop in hemoglobin and hematocrit levels were observed in normal rats given oral treatment with extracts of *A. cepa* and *A. sativum*, however, a statistically significant increase in red blood cells (RBC) compared to the control group was observed (Aletan, 2014). In another study evaluating the effects of the ethanol extracts of *J. curcas* on hematological parameters, HB, WBC and PCV were elevated at 300 mg/kg body weight, but the extract had no effect on the RBC concentration (Nwaka *et al*. 2015)

2.15 Knowledge gap

The review identified gaps on anthelmintic activity and acute toxicity of selected plants.

2.15.1 *Allium sativum*

Alkaloids, flavonoids, cardiac glycosides, terpenes and resins have been found in both cooked and uncooked plant (Gazuwa *et al.* 2013).

In vitro studies have shown that extracts of garlic have anthelmintic activity against trematodes and nematodes of Veterinary importance. However these extracts given to live goats and donkeys experimentally infected by helminthes were not effective. On the other hand freshly cut garlic was found to be effective against nematodes in mice. What could be the reasons why it is effective in mice and not in goats and donkeys? There could be a difference in the yield of the extract due to differences in extraction methods. Garlic has been studied for efficacy on important species of protozoa (*Trichomonas vaginalis*) and ticks (Hyalomma, Rhipicephalus and boophilus) and lice (Damalinia). In combination with Diminazene Aceturate garlic was studied for efficacy on *Trypanosoma brucei*.

These gaps presents an opportunities to try different extraction techniques, as well as investigate efficacy of garlic on helminthes of veterinary importance in other domestic species like dogs, cats

and pigs. More studies are also required for efficacy of garlic on Trypanosomes, protozoans causing GIT infections in domestic animals and other types of ectoparasites like fleas and mites.

For the acute toxicity studies, no visible signs were observed in rats given the test samples through the oral route however toxicity signs were observed in the rabbit given the test samples through the subcutaneous route. This suggests that the oral route reduces bioavailability of the potentially toxic elements. A study should be carried out on toxicity of *A. sativum* in other species of animals like the dog using both the oral and subcutaneous routes.

2.15.2 *Allium cepa*

Phytochemical screening tests done on this plant found the plant contain flavonoids, glycosides, saponins and tannins (Abhijeet *et al.* 2012).

In vitro studies have shown that crude extracts of *A. cepa* have anthelmintic activity. Studies in experimentally infected sheep have also shown that extracts of *A. cepa* combined with extracts of *Cocos nucifera* have marked anthelmintic activity against gastrointestinal nematodes and cestodes, however there is a need to investigate such activity for *A. cepa* extracts in isolation.

These gaps presents an opportunities to try different extraction techniques, as well as investigate efficacy of garlic on helminthes of veterinary importance in other domestic species like dogs, cats and pigs. More studies are also required for efficacy of garlic on Trypanosomes, protozoans causing GIT infections in domestic animals, other species of ticks and other types of ectoparasites like lice, fleas and mites.

Acute and sub-acute toxicity studies of *A. cepa* extracts given to rats orally found no visible signs or toxic effects. This presents an opportunity for this study to carry out acute toxicity studies of *A. cepa* using a different route in rats or other species.

2.15.3 *Jatropha curcas*

The phytochemical analysis of the methanol extract from leaves of this plant found the presence of alkaloids, cardiac glycosides, cyanogenic glycosides, phlobatannins, tannins, flavonoids and

saponins indicating that these active ingredients may have the potential for the treatment of diseases (Ebuehi and Okorie 2009).

Anthelmintic studies of seeds and leaves extracts of *J. curcas* have been done on nematodes of clinical importance in ruminants and poultry. However *in vivo* anthelmintic studies have only been done in poultry. This presents an opportunity to carry out *in vitro* and *in vivo* studies of *J. curcas* on helminthes of clinical importance to other domestic species like dogs, cats, pigs and horses/donkeys. Such studies could also investigate efficacy against trematodes and cestodes of clinical importance in all domestic species.

Studies of activity of *J. curcas* against ectoparasites have been done but only on ticks that are vectors in transmission of diseases of economic importance to domestic animals. This presents an opportunity for similar studies on other tick species as well as ectoparasites like fleas, mites and lice.

Studies of activity of *J. curcas* on protozoan parasites have been done but only on trypanosomes. This presents an opportunity for more studies on other protozoan species of Veterinary importance.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The study was conducted at the University of Nairobi, Faculty of Veterinary Medicine in Nairobi County, Republic of Kenya. The Country has an area of approximately 581 square kilometers, a population of over 44 million and it lies in the eastern part of Africa neighbouring Tanzania, Uganda, South Sudan, Ethiopia and Somali in the south, west, northwest, north and northeast respectively.

3.2 Collection and Identification of Plant materials

Different varieties of *A. sativum* and *A. cepa* are grown and marketed in all parts of the country for culinary uses. The Alliums were bought from the Mountain View Market situated 2kms from the University. Figure 1 and figure 2 are camera images of *A. sativum* and *A cepa* respectively.

Figure 1*: Image of *Allium sativum
(articles.granmasherbs.com)

***Figure 2*: Image of *Allium cepa* (www.123rf.com)**

J. curcas grows as shrub vegetation in Arid and Semi-Arid Lands parts of the country, the plant was collected from Ngong Forest of Ngong Hills in Kajiado County. The collected plant materials were taken to the Kenya National Museums Herbarium for identification. Figure 3 is an image of a *J. curcas* plantation showing the physical characteristics of the tree.

***Figure 3*: Image of *Jatropha curcas* plantation (www.chemicallygreen.com)**

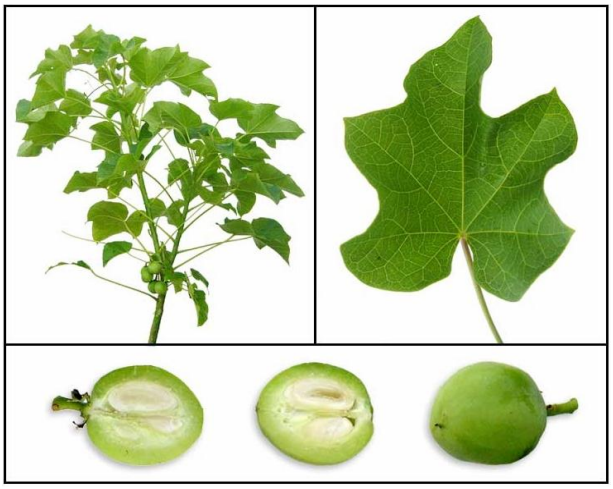

***Figure 4*: Illustration of leaves and seeds of *Jatropha curcas* (www.prota4u.com)**

3.3 Preparation of plant extracts

The bulbs of the Alliums and leaves of the *J. curcas* were oven dried separately at 60ºC for 48-72 hours. This was followed by grinding each of the dry plant materials in order to obtain fine powders which were sealed in a paper bag and kept in a cool dry place. Figures 5, 6 and 7 show dried plant materials of *A. sativum*, *A. cepa* and *J. curcas* respectively.

Figure 5: Dry bulbs of *Allium sativum* in an oven

Figure 6: Dry bulbs of *Allium cepa* in an oven

Figure 7: Dry leaves of *Jatropha curcas* in a paper bag

Figure 8: Grinding machine used to grind three dry plant materials

An aqueous extract of the leaves of *J. curcas* was obtained following the method described by Juliana *et al*., 2014. A total of 900 ml of distilled water was added to 100 g of the dry powder and the mixture was heated at 100^{o}C for 15 minutes. After cooling, the mixture was centrifuged then filtration was done using No.1 filter paper (M&N 615 12.5 CM) in order to separate the residues. The filtered solution was then freeze dried to obtain a light green colored powder with a recovery rate of 0.94% relative to dry powder. Figure 10 shows a research assistant weighing the ground powder of *J. curcas.*

Figure 9: Weighing of a powder of *Jatropha curcas*

The ethanol extract of leaves of *J. curcas* was prepared according to Ekundayo and Ekekwe (2013). One litre of 99.5% ethanol was mixed with 100 g of dried leaf powder and agitated several times for a period of 72 hrs. Filtration of the mixture was done using a Whatman No. 1 filter paper into a clean beaker and then evaporated over a sand bath at 80^{o}C. Drying was completed in an oven at 40^{o}C to prevent the dry extract from sticking to the walls of the container. This yielded a dark green (near black) colored paste with a recovery rate of 2.61%.

Aqueous extracts for both *A. sativum* and *A. cepa* were obtained as described by Mikhail (2009). A total of 100 g of dry powder from ground dry bulbs of each of the alliums was mixed with 900 ml of distilled water and heated in a water bath at 100^{o}C for 30 min. After cooling, filtration of the mixture

was done using Whatman No. 1 filter paper and the filtrate centrifuged before decanting the supernatant into a clean beaker. Figure 10 shows the mixture of *A. cepa* and water being filtered after extraction. The extracts were freeze dried, figure 11 shows aqueous extracts of *A. cepa* being freeze dried. *A. sativum* yielded a yellowish colored crystalline powder with a recovery rate of 18.25% while *A. cepa* yielded a sticky dark tan (dark brown) colored solid with a recovery rate of 16.75%.

Figure 10: Filtration of *Allium cepa* aqueous extract

Figure 11: Aqueous extract of *Allium cepa* mounted on a freeze drier

Ethanol extracts of the alliums were obtained according to Akintobi *et al.*, (2013). A total of 100 g of each powder was mixed with 400 ml of 99.5% ethanol and agitated several times for a period of 72 hrs. Figure 12 shows a mixture of *A. sativum* powder and ethanol in a conical flask. The mixture was filtered through a Whatman No. 1 filter paper into a clean beaker and then evaporated over a sand bath at 80^{o}C. Drying was completed in an oven at 40^{o}C to prevent the dry extract from sticking to the walls of the container. The *A. sativum* ethanol extract yielded a mid-brown colored paste with a recovery rate of 0.39% while the *A. cepa* ethanol extract yielded a maroon colored paste with a recovery rate of 2.03%. All the six extracts were stored in freezing temperatures (-18^{o}C).

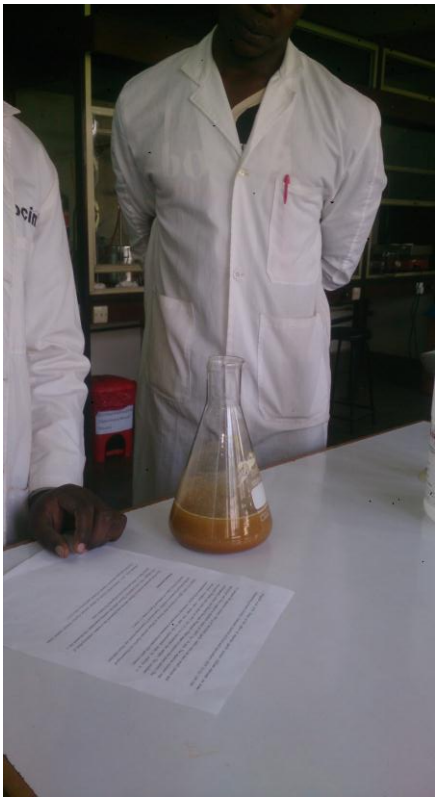

Figure 12: A mixture of 100 g of *Allium sativum* powder and ethanol

3.4 Study design

The study was designed as an experimental study which is also referred to as randomized controlled study. The experimental animals were allocated to three groups, exposed to treatment and then followed up under similar carefully controlled conditions. The study was carried out in two stages; Trial stage and experimental stage.

Trial stage

At this stage, the aqueous and organic solvent extracts of the three plants were tested *in vitro* for anthelmintic activity.

Determination of effects on hatching

This was done using the Egg Hatch Assay as described by Le Jambre (1976) and used by other researchers (Coles *et al.* 2006).

Determination of effects on survival of larvae (*Larval Motility Assay*)

This observation was only made on larvae of *A. caninum*. This is because they hatch into free larvae unlike those of *T. canis* which hatch within the egg shells making it difficult to observe their movements. Survival of larvae was determined after hatching by calculating the percentage of larvae that were motile (alive) and those that were not (dead) out of the total larvae that hatched (Thoithi *et al.* 2002).

Identification of the eggs

In vitro egg assay differentiated strongyle and ascarid eggs. *T. canis, T leonine, E. granulosa, T. ovis* and *T. hydatigena* lay ascarid type of eggs while *A. caninum, T. vulpis, S. lupi, D. caninum* and *D. immitis* lay strongyle type of eggs. Typical strongyle eggs have an ovoid shape and a thin wall (Figure 14). They measure about 40x65 micrometers, and contain already 4 to 16 cells when shed with the feces. Ascarid eggs are almost spherical, brown in color and measuring about 50x85 micrometers. They have a thick wall with a rough surface, and contain a single cell when in feces as illustrated in Figure 13. (www.parasitipedia.net)

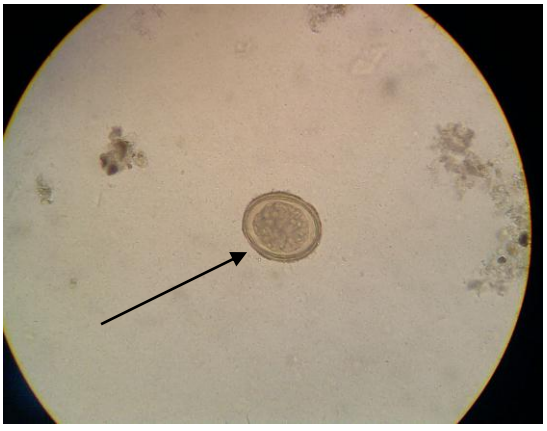

Figure 13: Ascarid egg

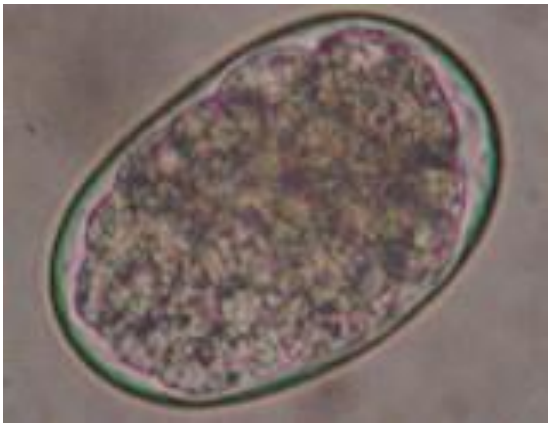

Figure 14: Strongyle egg

Preparation of the eggs solution

According to Coles *et al.* (2006) eggs intended for the hatching test should be used within 3 hrs of collection because sensitivity decreases with age of the eggs. To obtain fresh fecal samples, the puppies were given dog food pellets at 8 am on the day of harvesting, after starving them for 12-18 hrs, this ensures that they defecate within an hour of feeding releasing fresh samples.

Fecal floatation methods was used as described by (Byron *et al).* (2006). About 200 g of a pooled fecal sample was mixed into a suspension with saturated saline solution (360 g of NaCl in 1,000 ml of tap water) and allowed to settle for 30 minutes. A shallow, circular and transparent dish with a flat bottom was floated on the surface of the suspension for 10 minutes in order to allow the floating eggs to adhere to the bottom of the dish as illustrated in Figure 15. To recover the eggs, the bottom of the dish was washed off using distilled water into a clean beaker then centrifuged twice in order to remove the excess salt. 15 ml of distilled water was added to the final residue and mixed well. 50 *u*l of this solution were dropped into three microscope slides and a cover slip placed onto each and then observed under a light microscope at X10 magnification in order to count the number of strongyle and ascarid eggs in each slide. The average estimated number of eggs per 50 *ul* of the parasite egg solution was 173 strongyle and 27 ascarid eggs.

Figure 15: A plastic petri dish floating on a mixture of concentrated salt solution and fecal material

Exposing the eggs to the plant extracts

The extracts were reconstituted by adding 40 mg of each dry extract into 2 ml of distilled water in order to make a stock solution. The aqueous extracts of each of the plant was dissolved readily in water while the ethanol extracts of *A. sativum* and *A. cepa* dissolved after raising the temperature of the mixture in a water bath at 40^{o}C for 15 minutes and shaking vigorously. The ethanol extract of *J. curcas* could not dissolve in water even after shaking or raising the temperature. Therefore it was not used for any tests.

The stock of 20,000 ug/ml of each plant extract was diluted serially to give 10,000 ug/ml, 5,000 ug/ml, 2,500 ug/ml, 1,250 ug/ml, 625 ug/ml, 312.5 ug/ml and 156.25 ug/ml in a microtitre plate in order to obtain 150 *ul* of each dilution per well. A total of 50 *ul* of the parasite solution was added into each well to make a final mixture of 200 ul of parasite eggs and extract in each well. Further dilutions of 78.13 ug/ml, 39.06 ug/ml, 19.53 ug/ml, 9.77 ug/ml, 4.88 ug/ml, 2.44 ug/ml, 1.22 ug/ml and 0.61 ug/ml were done for ethanol extracts of both *A. cepa* and *A. sativum* and for the aqueous

extract of *A. cepa*. For the positive control a similar dilution was made as for the drug preparation (Vermic Total^{TM}) containing febantel and pyrantel permoeate which are both effective on *A. caninum* and *T. canis*. For the negative controls, one well had 200 ul of the parasite egg solution and another one had 150 *ul* of 99.5% ethanol mixed with 50 *ul* of the parasite egg solution. The microtitre plate cover was replaced, wrapped up with aluminum foil and incubated for 2 weeks. Observations were made under an inverted microscope after 48 hrs and again after 96 hrs for the strongyle eggs then on alternate days up to 2 weeks for the ascarid eggs. This is because strongyle eggs usually hatch after 48-72 h into free larvae while ascarid eggs develop to a fully larvated egg over a period of 2-6 weeks and does not break into free larvae (Azam *et al.* 2012). The readings were done by counting the number of free larvae and the number of developing ascarid eggs. Inhibition percentages were calculated using the formula below as used by Cala *et al.* (2012). L1 in the formula represents the number of free larvae

$$E = \frac{(Eggs + L1) - L1 \times 100}{Eggs + L1}$$

Experimental stage

The most effective extracts from the trial stage (ethanol extract of *A. cepa*) were tested for anthelmintic efficacy *in vivo.*

Experimental animals

Fifteen (15) mongrel puppies of mixed sexes, aged between 8 and 10 weeks, with an average weight of 2.2 kg and showing signs of natural helminth infection were purchased from households in Ndumboini village in Kabete. The puppies were vaccinated against parvovirus infection 7 days before admission to the small animal clinic at the Faculty of Veterinary Medicine of the University of Nairobi where they were housed and fed in dog kennels during the experiment. Screening for

helminth infection was done by microscopic examination of fecal smears from each puppy. Only puppies with helminth infestations were used for the experiment.

Acute toxicity test of the ethanol extract of *A. cepa*

The purpose of acute toxicity testing of the ethanol extract of *A. cepa* was to establish whether the concentration of the extract found effective *in vitro* will have any toxicity in the experimental puppies if administered orally. This was done using the methods described in the OECD guidelines 432 (adopted on 17th December 2001) for testing of chemicals under the acute oral toxicity-acute toxic class method, with a starting dose of 300mg/kg of body weight. This method uses 3 animals of the same sex for each step, the method is reproducible and gives similar results to those of other acute toxicity testing methods. Rats were used as experimental animals for this test. The weight of each animal was used to calculate the concentration of the extract to be administered orally in 2 ml of distilled water to achieve the recommended dosage in the guideline.

Determination of *in vivo* anthelmintic activity

This was done using the fecal egg count reduction test (Coles *et al*., 2006). A total of 15 puppies were grouped into three groups of 5 animals each. Group 1 was treated with the extract, group 2 was a positive control given a dose of a commercial anthelmintic (Vermic Total^{TM}) used for treatment of helminthosis in dogs while group 3 was a negative control given distilled water.

The lowest concentration of the ethanol extract found to be effective against strongyle and ascarid eggs from trial stage was 1,250 ug/ml in distilled water. The oral route of administration was selected for administering the extract and distilled water, each animal being given 10 ml. For the positive control group (2) each animal was given half a Tablet of Vermic Total^{TM} orally. Fecal samples (fresh droppings) were obtained from each puppy a day before treatment (day 0) and six different days post treatment (days 1, 3, 5, 7, 10 and 14) for determination of the number of helminth eggs per gram (EPG) of feces using the Mc Master technique detailed by Coles *et al*. (2006). The anthelmintic effects were established by computing the percentage reduction in fecal egg count (%FECR) using

the pretreatment and post treatment EPG counts. The % FECR value was corrected for changes in the control groups using the formula; %FECR= {1 (T2/T1 X C1/C2)} X 100. Where T and C are the arithmetic means for the treated and control groups and the subscripts 1 and 2 designate the counts before and after treatment respectively (Mbaria *et al*. 2006).

Determination of hematological changes

1 ml of whole blood for hematology was collected from each puppy and analyzed using a hemogram on days 0, 7 and 14 to determine changes in the hematological parameters. White Blood Cells (WBC), Red Blood Cells (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) were analyzed.

Pathological changes

Two puppies from each group were then randomly selected and sacrificed and carefully dissected to examine for any pathological changes as well as collect intestinal contents to quantify and identify adult worms using morphological characteristics.

3.5 Statistical data analysis

The mean, range and standard deviation of the fecal egg counts and hematological parameters were determined. The pretreatment and post treatment EPG values as well as White Blood Cells (WBC), Red Blood Cells (RBC), Hemoglobin (HGB), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Hemoglobin Concentration (MCHC) values for each animals were entered into the statistical package SPSS from where the statistical analysis was done. An independent-sample T test was used to compare the means of the treatment and control groups while a paired-sample T test was used to compare pretreatment and post treatment means of each group.

3.6 Ethical considerations

Approval of the study was given by the University of Nairobi Biosafety, Animal Use and Ethics committee. The study applied principles that reduced the number of animals used and reduced pain and distress to animals. Information from the study was handled with confidentiality.

CHAPTER FOUR

RESULTS

4.1 Introduction

The results of the *in vitro* experiment are presented in the first section of this chapter. *A cepa* was found to be the most effective extract from the in vitro experiment results and therefore was the only extract used for the *in vivo* experiment for puppies whose results are presented in the second section of this chapter.

4.2 Results of the trial experiment

Figures 16 and 17 illustrate a negative control and a dose response of ethanol extract of *A. cepa*. Figure 16 is a microscopic view of a negative control, free larvae from hatched strongyle eggs and developing ascarid eggs are seen. Figure 17 is a microscopic view of a mixture of the egg solution and ethanol extract of *A. cepa* at 156 ug/ml, the free larvae are seen to have died, a strongyle egg appears inhibited while the ascarid eggs are not developing. Figure 18 is a microscopic view showing typical development of a *T. canis* egg. Figure 19 is a microscopic view of a positive control showing inhibition of hatching and development of both strongyle and ascarid eggs respectively.

Table 1 shows the percentage inhibition of egg hatching and effects on motility of larvae of each of the six extracts on strongyle eggs. Table 2 shows the percentage Egg Hatch Inhibition on ascarid eggs and Table 3 shows the dose response on further dilution of aqueous and ethanol extracts of *A cepa* and ethanol extract of *A. sativum*. The aqueous and ethanol extracts of *A. cepa* inhibited hatching and development of strongyle and ascarid eggs respectively as shown in Tables 1 and 2. The aqueous extract of *A. cepa* had moderate effect on survival of larvae while the ethanol extract of *A. cepa* appeared to kill all larvae as shown in Table 1. However, the ethanol extract gave higher percentage inhibition rates at lower concentrations than the aqueous extract. This suggests that there was a better dose response for the ethanol extract of *A. cepa*. Ethanol extract of *A. sativum* also inhibited hatching of eggs of both parasites and had moderate effect on survival of larvae as shown

in Table 3. Both aqueous extracts of *A. sativum* and *J. curcas* did not have effect on the eggs and on survival of larvae as shown in Tables 1 and 2. None of the extracts was as active as the combination drug VERMIC TOTAL^{TM} at equal concentrations as shown in Table 4 where the drug caused 100% inhibition of egg hatch, egg development.

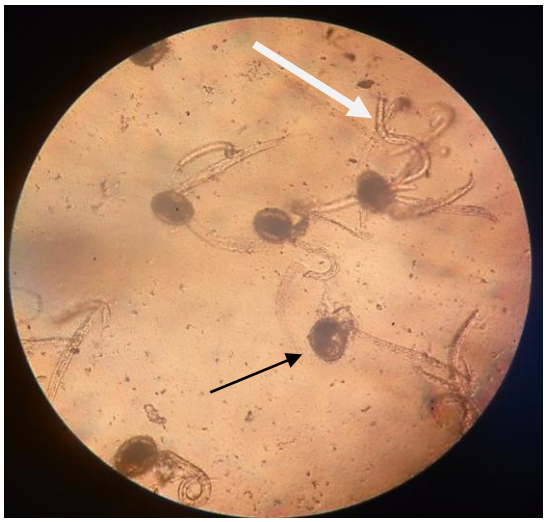

Figure 16: Free larvae (white arrow) and developing ascarid eggs (black arrow)

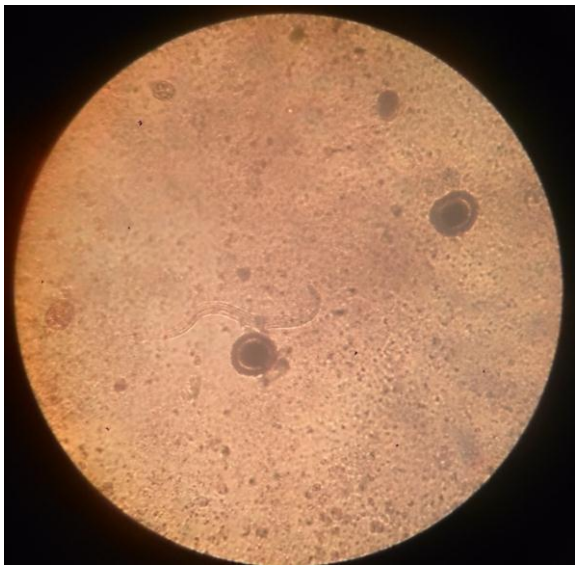

Figure 17: Dose response of ethanol extract of *Allium cepa* at 156 ug/ml

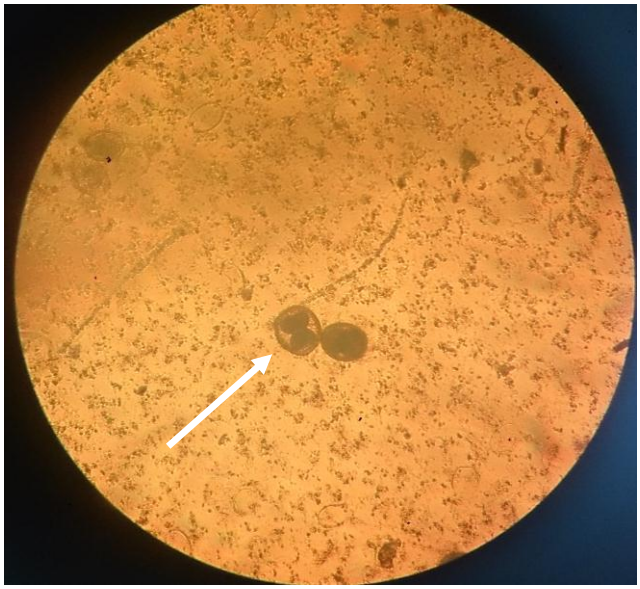

Figure 18: Typical view of developing *Toxocara canis* eggs (white arrow)

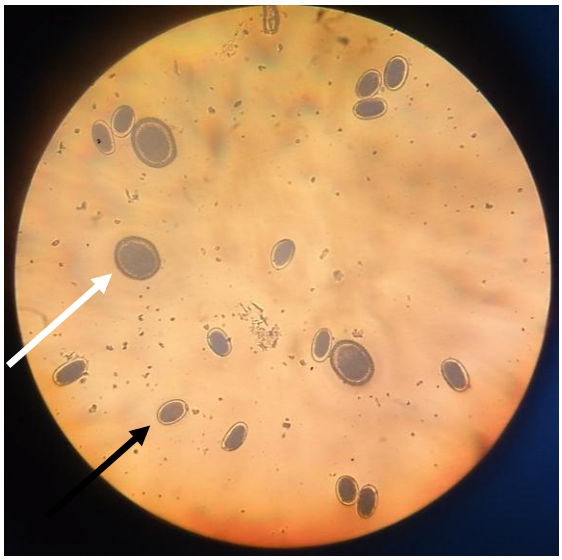

Figure 19: Inhibited hatching of strongyle egg (Black arrow) inhibited development of ascarid egg (white arrow)

Table 1: Anthelmintic activity of water and ethanol extracts of bulbs of *Allium cepa, Allium sativum* and leaves of *Jatropha curcas* against strongyle eggs.

Concentration (ug/ml)	**Plant extract Eggs Hatch Inhibition (EHI) and Larval Motility Assay (LM)**									
	Allium cepa				***Allium sativum***				***Jatropha curcas***	
	Aqueous (%)		**Ethanol (%)**		**Aqueous (%)**		**Ethanol (%)**		**Aqueous (%)**	
	EHI	LM	EHI	LM	EHI	LM	EHI	LM	EHI	LM
10,000	99	70	100	0	13	93	99	0	11	94
5,000	70	79	100	0	9	90	100	0	7	97
2,500	63	77	100	0	8	92	98	0	8	99
1,250	53	84	97	0	4	95	99	0	5	100
625	24	88	89	0	4	97	92	0	4	99
312.5	27	93	86	0	3	97	85	15	4	100
156.25	15	95	80	0	3	100	78	17	2	100

Table 2: Anthelmintic activity of water and ethanol extracts of bulbs of *Allium cepa, Allium sativum* and leaves of *Jatropha curcas* against ascarid eggs.

Concentration (ug/ml)	**Plant extract Eggs Development Inhibition (EDI) *on ascarid eggs***				
	Allium cepa		***Allium sativum***		***Jatropha curcas***
	Aqueous (%)	**Ethanol (%)**	**Aqueous (%)**	**Ethanol (%)**	**Aqueous (%)**
	EDI	EDI	EDI	EDI	EDI
10,000	78	100	8	90	5
5,000	54	100	7	89	4
2,500	50	100	7	88	5
1,250	43	100	6	82	3
625	4	90	6	79	3
312.5	3	88	5	75	2
156.25	0	80	3	70	2

Table 3: Anthelmintic activity of ethanol extracts of bulbs of *Allium cepa,* and *Allium sativum* against strongyle and ascarid eggs and against larvae on further dilution

Concentration (ug/ml)	**Percentage Egg Hatch/Development Inhibition**				**Percentage Larval Motility**	
	Strongyle		***Ascarid***		***Larvae***	
	Allium cepa	***Allium sativum***	***Allium cepa***	***Allium sativum***	***Allium cepa***	***Allium sativum***
78.13	70	78	72	68	0	28
39.06	64	69	65	59	2	32
19.53	59	51	57	43	5	38
9.77	49	36	42	28	12	46
4.88	38	23	31	15	25	56
2.44	23	14	18	10	40	76
1.22	12	5	7	9	51	80
0.61	3	2	4	8	55	83

Table 4: Percentage egg hatch inhibition, egg development inhibition and larvae mortality of Vermic Total™ used as positive control on strongyle and ascarid eggs

Concentration (ug/ml)	Percentage egg hatch, egg development and larvae motility assay		
	Strongyle eggs		Ascarid eggs
	EHI	LM	EDI
	Vermic Total	Vermic Total	Vermic Total
10,000	100	0	100
5,000	100	0	100
2,500	100	0	100
1,250	100	0	100
625	100	0	100
312.5	100	0	100
156.25	100	0	100
78.13	100	0	100
39.06	100	0	100
19.53	100	0	100
9.77	100	0	100
4.88	100	0	100
2.44	100	0	100
1.22	100	0	100
0.61	100	0	100

Key:

EHI Egg Hatch Inhibition

LM Larvae Motility

EDI Egg Development Inhibition

Figure 20 is a graph plotting the percentage inhibition of hatching of the stronyle eggs by both the ethanol extracts of *A. cepa* and *A. sativum* from 0.61 ug/ml and 78.13 ug/ml of each. The y axis plots the parentages of inhibition while the x axis plots the corresponding concentrations of the plant extracts (rounded off to the nearest whole digit). It shows that as the dose increases the percentage inhibition also increases for both extracts. There is a dose response which is higher for *A. cepa* up to a concentration of 39 ug/ml

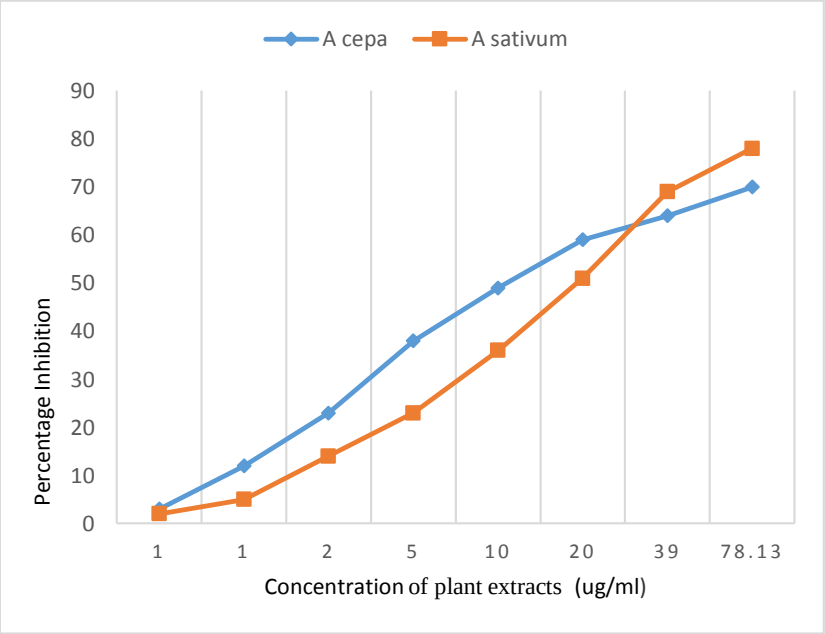

Figure 20: Dose response curve showing the Egg Hatch Inhibition of ethanol extracts of bulbs of *Allium cepa* and *Allium sativum* on stronyle eggs.

Figure 21 is a graph plotting the percentage inhibition of egg development of ascarid eggs by both the ethanol extracts of *A. cepa* and *A. sativum* from 0.61 ug/ml and 78.13 ug/ml of each. The y axis plots the parentages of inhibition while the x axis plots the corresponding concentrations of the plant extracts (rounded off to the nearest whole digit). It shows that as the dose increases the percentage inhibition also increases for both extracts. There is a dose response which is higher for the ethanol extract of *A. cepa.*

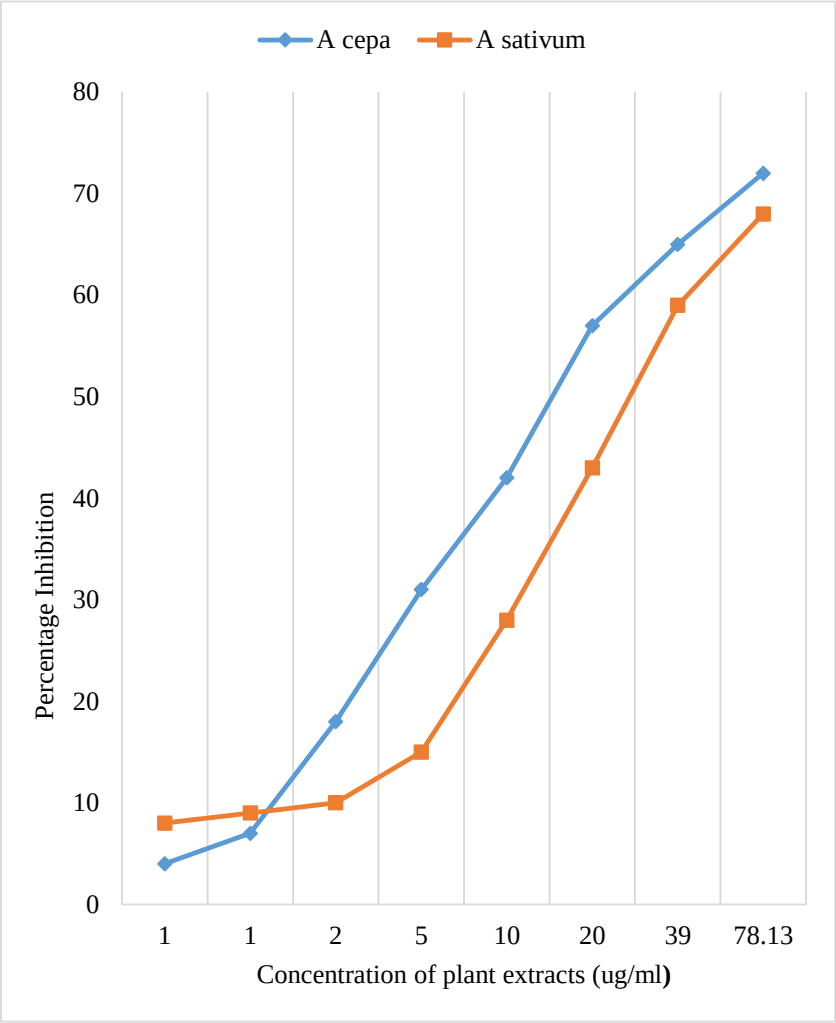

Figure 21: Dose response curve showing the Egg Development Inhibition of ethanol extracts of bulbs of *Allium cepa* and *Allium sativum* on development of ascarid eggs.

4.3 In vivo results

The fecal Egg count Per Gram (EPG) for the puppies before and after treatment for strongyle and ascarid eggs are shown in Table 5 and Table 6.

Table 5: Strongyle eggs per gram of feces (n=5) in a study on the efficacy of *A. cepa* ethanol extract against mixed gastrointestinal helminth infestations in puppies

Groups	Animal ID	DAYS						
		Pretreat	Post Treatment					
		D0	D1	D3	D5	D7	D11	D14
Group 1								
Extract	G102	750	5,700	3,100	700	300	960	555
	G103	#	11,950	1,650	500	5,700	1,090	#
	G104	3,050	#	#	#	2,100	2,600	1,045
	G105	6,050	6,400	7,250	8,400	1,500	1,350	1,450
	G106	5,650	#	7,450	8,500	2,150	0	#
Group 2	G207	1,050	1,500	0	0	0	0	0
Positive	G208	3,450	0	0	0	0	0	0
control	G209	550	#	0	0	#	#	0
	G210	6,350	0	#	#	#	1,850	0
	G211	#	0	0	0	0	0	0
Group 3	G313	6,550	6,900	7,250	6,800	#	7,400	7,750
Negative	G314	#	8,550	9,000	8,900	9,150	9,500	#
control	G315	2,450	2,500	2,650	2,800	#	2,700	3,350
	G316	1,300	2,200	2,750	3,450	3,150	3,350	3,750
	G317	600	1,150	1,950	2,500	2,500	#	3,100

Key: # indicates no sample was collected from the animal

Table 6*:* Ascarid eggs per gram of feces (n=5) in a study on the efficacy of *A. cepa* ethanol extract against mixed gastrointestinal helminth infestations in puppies

Groups	Animal ID	DAYS Pretreat	Post treatment					
		D0	D1	D3	D5	D7	D11	D14
Group 1								
Extract	G102	0	50	50	50	0	55	50
	G103	#	550	350	150	200	130	#
	G104	350	#	#	#	0	0	0
	G105	0	400	0	150	50	0	0
	G106	0	0	0	100	50	#	0
Group 2	G207	0	50	0	0	0	0	0
Positive	G208	200		0	0	0	0	0
control			0					
	G209	50	#	0	0	#	#	50
	G210	200	150	#	#	#	0	0
	G211	#	250	0	0	0	0	0
Group 3	G313	150	0	200	0	#	0	0
Negative	G314	#	0	0	310	350	0	#
control	G315	165	160	450	0	#	650	0
	G316	0	0	0	0	150	0	0
	G317	0	0	0	0	0	#	150

Key: # indicates no sample was collected from the animal

The pretreatment and post treatment group means (averages) and ranges for EPG of strongyle and ascarid eggs are shown in Tables 7 and 8.

Table 7*:* Group means and ranges of strongyle eggs per gram of feces (n=5) in a study on the efficacy of *A. cepa* ethanol extract against mixed gastrointestinal helminth infestations in puppies

Groups		D0	D1	D3	D5	D7	D11	D14
G1	Mean	8,750	8,238	4,630	4,120	2,350	1,550	1,017
	Range	750-6050	5,700-11,950	1,650-7,450	700-8,500	300-5,700	960-2,600	555-1,450
G2	Mean	2,850	1,500	0	0	0	0	0
	Range	550-6,350	0-1500	0	0	0	0	0
G3	Mean	2,725	4,260	4,720	4,890	4,933	5,738	4,488
	Range	600-6,550	1,150-6,900	1,950-9,000	2,500-8,900	2,500-9,150	2,700-9,500	3,100-7,750

Table 8*:* Group means and ranges of ascarid worm eggs per gram of feces (n=5) in a study on the efficacy of *A. cepa* ethanol extract against mixed gastrointestinal helminth infestations in puppies

Groups		D0	D1	D3	D5	D7	D11	D14
G1	Mean	87.5	250	100	112.5	60	46.25	12.5
	Range	0-350	50-400	50-350	50-150	50-200	55-130	0-50
G2	Mean	112.5	112.5	0	0	0	0	0
	Range	50-200	50-250	0	0	0	0	0-50
G3	Mean	78.75	32	130	62	166.67	162.5	37.5
	Range	150-165	0-160	200-450	0-310	150-350	0-650	0-150

Three days after treatment, hookworm EPG counts in the treatment group had dropped significantly ($p<0.05$) while there was no significant drop in the post treatment ascarid worm EPG counts. A statistically significant difference ($p<0.05$) was observed in post treatment hookworm EPG counts 7 days after treatment in the negative control group while the statistical difference in the post treatment ascarid worm EPG counts was not significant. A statistically significant difference ($p<0.05$) was observed in the treatment and control group means for post treatment hookworm EPG values on the 5^{th} day after treatment, and on the 1^{st} day after treatment for ascarid worm EPG values.

The percentage fecal egg count reduction was 47% for hookworm eggs and a negligible reduction in ascarid worm eggs. No signs of toxicity or behavioural changes were observed in puppies after oral administration of the *A. cepa* ethanol extract at 6 mg/kg. Further studies should determine if an increased dosage would have more efficacy, refine the extract to isolate the active chemical compounds and to establish the mode of action of the chemical compounds.

Acute toxicity test

In the first step using a dose of 300mg/ml, none of the rats died or showed any signs of acute toxicity. In the second step using a dose of 2000 mg/ml, none of the rats died or showed any signs of acute toxicity. The outcome puts the extract in category 5 with an LD_{50} cut off of between 2000 mg/kg and 5000 mg/kg of body weight as illustrated in Appendix 3.

Hematology

Table 9 shows the p values from comparison of the arithmetic means of the monitored hematology parameters between the treatment and negative control groups. The p values were obtained using the levene's test of significance. Table 10 shows the p values from comparison of the arithmetic means of the monitored hematology parameters between the treatment and positive control groups using the same method.

Table 9*:* P values from a Levene's test of significance, comparing means of treatment (Group 1) and negative control (Group 3) groups in a study on the efficacy of *A. cepa* ethanol extract against mixed gastrointestinal helminth infestations in puppies

Parameter	**Sampling days (p values)**		
	D0	D7	D14
WBC	0.164	0.006	0.437
RBC	0.406	0.050	0.270
HGB	0.815	0.035	0.857
HCT	0.440	0.025	0.255
MCV	0.112	0.534	0.860
MCH	0.802	0.980	0.032
MCHC	0.172	0.809	0.639

Table 10*: P* values from a Levene's test of significance, comparing means of treatment (Group 1) and positive control (Group 2) groups in a study on the efficacy of *A. cepa* ethanol extract against mixed gastrointestinal helminth infestations in puppies

Parameter	Sampling days (p values)		
	D0	D7	D14
WBC	0.418	0.004	0.478
RBC	0.338	0.286	0.867
HGB	0.08	0.786	0.509
HCT	0.365	0.249	0.892
MCV	0.008	0.288	0.144
MCH	0.174	0.269	0.280
MCHC	0.768	0.013	0.893

Post Mortem Examination

The sacrificed animals were extremely emaciated and anemic with foci of congestion and hemorrhages in the intestinal mucosa. Adult worms were isolated from their intestinal lumen. The number of adult worms isolated from animals in group 2 were fewer as compared to animals from group 1 and group 3 in some, while in others no adult worms were found. This shows that the commercial anthelmintic expelled more adult worms than the extract. Figures 22 shows round worms (ascarids, hookworms and whip worms) and Figure 23 shows *Dipylidium caninum* worms recovered from intestinal lumen of one of the animals sacrificed from group 1.

Figure 22: Asacrids, hookworms and whip worms

Figure 23: *Dipylidium caninum* with its characteristic elongated oval segments

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This is the first time the anthelmintic efficacy of the aqueous and ethanol extract of bulbs of *A. cepa* and *A. sativum* as well as the leaves of *J curcas on A. caninum* and *T. canis* have been reported in dogs. The results indicate that the ethanol extracts of *A. cepa* and *A. sativum* have anthelmintic properties against strongyle eggs and ascarid eggs *in vitro*. There was a 100% inhibition of Egg Hatching and Egg Development up to a dilution of 1,250 ug/ml for the *A. cepa* ethanol extract after which a dose response was observed. This percentage inhibition was higher compared to that of the ethanol extract of *A. sativum* suggesting that the *A. cepa* extract has a higher anthelmintic efficacy. Both extracts showed good anthelmintic activity since they affected the survival of larvae. However, none of these extracts was as active as Vermic Total™ (Febantel and Pyrantel Permoate). The aqueous extract of *J. curcas* had no effect in terms of inhibition of egg hatching or development but killed the larvae in a dose dependent way. The ability to affect both eggs and larvae as shown by the ethanol extracts of *A. cepa* and *A. sativum* as well as the aqueous extract of *A. cepa*, is comparable to that of commonly used anthelmintic drugs like benzimidazoles which affect all stages of nematode worms (Behm *et al.* 1983). The results agree with reports from similar research work which reported that crude extracts of *A. cepa* and *A. sativum* have anthelmintic properties (Abhijeet *et al.* 2012; Mantawy *et al.* 2012; Kumar and Dinesh 2014; Zafar *et al.* 2014).

Higher concentrations of aqueous extracts of *A. sativum* and *J. curcas* may show better effect on egg hatching and survival of larvae. The use of 20,000 ug/ml as the concentration of stock solutions for serial dilution of each extract was based on methods described by Thoithi *et al*. (2002). More work can be done to test the *in vitro* effects of these extracts at higher concentration ranges.

Dimethyl Sulfoxide (DMSO) or Tween80 can be used to concentrate organic solvent dry extracts which cannot be reconstituted in water. However according to Luciana *et al.* (2011) worms can

tolerate up to 2% DMSO or Tween 80 although even these solvents at 2% cannot dissolve extracts made with pure ethanol. Therefore this study did not use DMSO or Tween 80 to reconstitute the ethanol extract of *J. curcas* but opted to remove it from the experiment.

Further pharmacological work should be carried out on the efficacy of the ethanol extracts of *A. cepa* and *A. sativum* in puppies to determine the toxic effects in mice in order to get further information that can support the use of the herbal medicines for control and treatment of worm infestations in dogs.

The results of the acute toxicity study of the ethanol extract of *A. cepa* in rats, found the LD_{50} to be between 2000 mg/kg and 5000 mg/kg of body weight. This finding is similar to those of Shenoy et al. (2009) which reported that the LD_{50} of extracts of *A. cepa* to be 3,000 mg/kg of body weight in rodents. This provided a wider safety margin suggesting that it was safe to administer the extract at 6 mg/kg of body weight.

The results obtained following oral administration of the ethanol extract of *A. cepa* at 6 mg/kg of body weight in puppies indicate that the extract has anthelmintic effect against strongyle worm infestations in puppies. The 47% reduction in EPG counts of hookworm eggs was attributed to the effects of the extract demonstrating that administration of the extract reduced hookworm infestation in the puppies. However, there was no reduction in the EPG counts of the ascarid worm eggs after giving the extract 'per os' as expected from the findings from the *in vitro* study on the extract on *T. canis*. Even though, no studies have reported the *in vivo* anthelmintic efficacy of the crude ethanol extract of *A. cepa* in isolation or in combinations, some studies have reported anthelmintic activity of the plant. *A. cepa* combined with *Cocos nucifera* taken in food stopped gastrointestinal infections in sheep (Heinz, 2010). The same combination was found to have marked activity against adult *Trichuris muris* and adult *Hymenolepis microstoma* in mice

The results show that the 47% efficacy against hookworms observed in treated puppies may have been due to the anthelmintic properties of the crude ethanol extract of *A. cepa*. This is supported by

the hematological changes that occurred as a result of administration of the extract. There was a significant drop in WBC ($p=0.035$) 7 days after treatment and a significant increase in RBC ($p=0.04$) and HGB ($p=0.001$) 14 days after treatment. The changes in hematological parameters when compared between the treatment and control groups were significant ($p<0.05$) 7 days after treatment for WBC, RBC, HGB and HCT, and 14 days after treatment for MCHC. These hematological changes may show that administration of the extract reduced worm infestations in the puppies. The increase in RBC and decrease in WBC as observed may be due to a decrease in worm burden. These findings agree with those from a study on changes of hematological parameters due to worm infestations by Lefkaditis *et al.* (2009). The normal hematological parameters in dogs as reported by Research Animal Resources of the University of Minnesota (Appendix 4) were used for comparison with the observed values in dogs.

5.2 Conclusions

The following conclusions were made from the study.

1. All the six extracts tested *in vitro* were found to have some anthelmintic activity and the most effective was the ethanol extract of *Allium cepa* causing 100% inhibition of egg hatching at 1,250 ug/ml.
2. The ethanol extract of *Allium cepa* may be selective in its *in vivo* anthelmintic efficacy causing a 47% drop of strongyle eggs shed in feces and having no significant drop of ascarid eggs shed in feces.
3. None of the extracts was observed to have efficacy that can be compared to that of Vermic Total^{TM} which is an anthelmintic drug used as a positive control in the experiment.
4. The hematological changes in RBC and WBC observed in the treatment group may be suggestive that administration of the ethanol extract of *A. cepa* reduced the worm burden. These changes were statistically significant ($p=0.006$ for WBC and $p=0.050$ for RBC)

compared to those of the negative control group and therefore attributed to the effects of the extract.

5.3 Recommendations

Efficacy studies should be done in order to test whether higher concentrations of the plant extracts can achieve a higher percentage inhibitions of egg hatching *in vitro* and higher reduction of fecal worm egg counts *in vivo*. The studies can be repeated in order to check if repeated treatments and graded doses *in vivo* will be more effective. The wide safety margin of the ethanol extract of *A. cepa* as determined from the acute toxicity tests indicates that the experiment can be repeated using higher dose ranges and repeated doses.

Further studies are required in order to establish which components of the crude plant extracts are responsible for the observed anthelmintic effects.

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APPENDICES

Appendix 1: Letter of approval from the Biosafety, Animal Use and Ethics Committee of the University of Nairobi

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

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

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

Dr Kenneth Otieno Orengo
c/o Dept of PHPT

8/06/2015

Dear Dr Orengo,

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

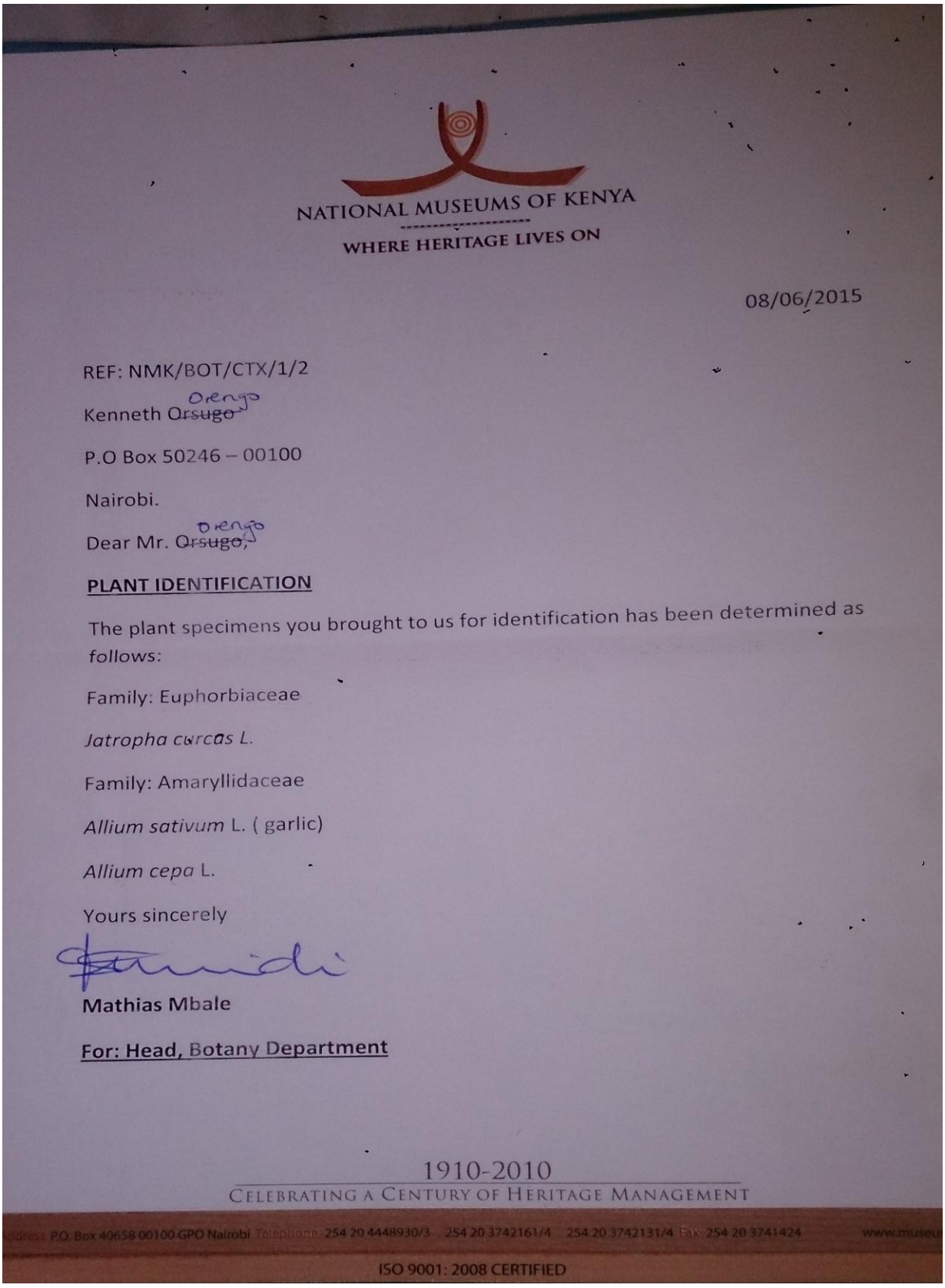
Efficacy of *Allium sativum*, *Allium cepa* and *Jatropha curcus* on *Toxocara canis* in dogs and acute toxicity in mice
By Dr Kenneth Otieno Orengo J56/67416/2013

We refer to the above proposal that you submitted to our committee. We have now reviewed your proposal and have noted that you have satisfactorily addressed issues to do with humane care and treatment of animals namely; dogs and mice, to be used in the study. Furthermore, the proposed numbers of experimental animals are within acceptable limits.

We therefore approve your work as per your proposal

Rodi O. Ojoo BVM, M.Sc, Ph.D
Chairman, Biosafety, Animal Use and Ethics Committee,
Faculty of Veterinary Medicine

Appendix 2: Plant identification by the botany section of the National Museums of Kenya



NATIONAL MUSEUMS OF KENYA

WHERE HERITAGE LIVES ON

08/06/2015

REF: NMK/BOT/CTX/1/2

Kenneth ~~Orsugo~~ Orengo

P.O Box 50246 – 00100

Nairobi.

Dear Mr. ~~Orsugo,~~ Orengo

PLANT IDENTIFICATION

The plant specimens you brought to us for identification has been determined as follows:

Family: Euphorbiaceae

Jatropha curcas L.

Family: Amaryllidaceae

Allium sativum L. (garlic)

Allium cepa L.

Yours sincerely

Mathias Mbale

For: Head, Botany Department

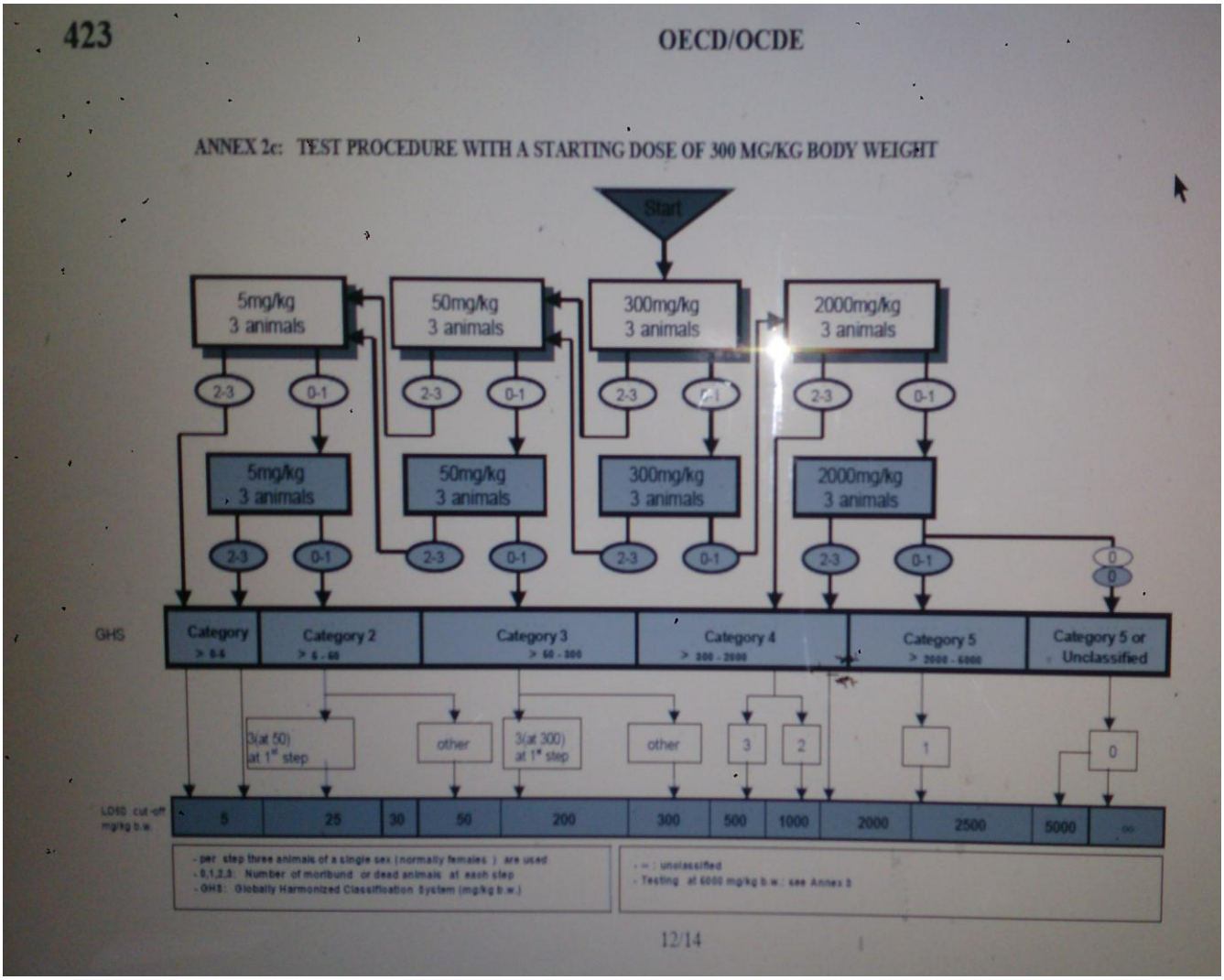
1910-2010

CELEBRATING A CENTURY OF HERITAGE MANAGEMENT

Address: P.O. Box 40658 00100 GPO Nairobi Telephone: 254 20 4448930/3 254 20 3742161/4 254 20 3742131/4 Fax: 254 20 3741424 www.museu

ISO 9001: 2008 CERTIFIED

Appendix 3: OECD guideline 423 annex 2c: Test Procedure with a starting dose of 300 mg/kg body weight



Appendix 4: Reference values for normal hematological parameters in Animals

	Dog	Cat	Rhesus	Baboon	Swine	Sheep	Cow	Rabbit	G Pig	Hamster	Rat	Mouse	Gerbil
PCV (%)	29-55	25-41	26-48	33-43	32-50	24-45	24-48	30-50	37-48	40-61	36-54	39-49	43-60
Hgb (g/dl)	14.2-19.2	14.2-19.2	8.8-16.5	10.9-14.3	10-16	8-16	8-15	10-15	11-15	10-18	11-19.2	10.2-16.6	12.6-16.2
MCV (fl)	65-80	65-80	72-86	71.2-82.8	50-68	23-48	40-60		78-95	67-77	48-70	41-49	
MCH (pg)	12.2-25.4	12.2-25.4	18.5-36.6	23.5-27.1	17-23	8-12	11-17						
MCHC (g/dl)	32-36	32-36	25.6-40.2	31.6-34.2	30-36	31-38	30-36		27-37	30-34	40		
WBC (x1000)	5.9-16.6	3.8-19	7-13	3.8-15.5	7-20	4-12	4-12	7-13	4.5-11	5-8.9	6-18	6-15	7-15
Diff. (%)													
segs	51-84	34-84	20-56	32-90	28-50	20-40	20-40	20-60	28-44	17-30	10-30	10-40	5-34
bands	0-4	0-1	0-6	0-1	0-10	0-2	0-4						
lymphs	8-38	7-60	40-76	9-63	40-60	40-70	40-70	40-80	39-72	50-81	65-85	55-95	60-95
monos	1-9	0-5	0-11	0-5	2-10	0-6	1-6	1-4	2-6	0-3	0-5	1-4	0-3
eos	0-9	0-12	0-14	0-3	0-10	0-10	0-4	0-4	0-5	0-4	0-6	0-4	0-4
basos	0-1	0-2	0-6	0-1	0-2	0-3	0-2	1-7	0-3	0-1	0-1	0-1	0-1
plat(x1000)	160-525	160-660	109-597	151-481	120-720	100-800	50-750	125-270	250-850	200-500	500-1300	160-410	400-600
Fibrinogen (mg/dl)	200-400		118-214	100-500	100-500	300-700		263-572	200-300				

Key:

eos:	Eosinophils	Plat:	Platelets
Basos:	Basophils	G pig:	Guinea Pig
Segs:	Mature Neutrophils	Bands:	Immature Neutrophils