

TITLE PAGE

**EVALUATION OF THE TOXICITY AND ANTHELMINTHIC EFFECT OF
METHANOL EXTRACT OF *Duranta erecta* FRUITS**

**A PROJECT REPORT SUBMITTED TO THE DEPARTMENT OF VETERINARY
PARASITOLOGY AND ENTOMOLGY, FACULTY OF VETERINARY MEDICINE,
UNIVERSITY OF NIGERIA, NSUKKA IN FULFILMENT OF THE
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN
VETERINARY PARASITOLOGY**

BY

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(PG/MSC/12/62314)

JANUARY 2017

DEDICATION

This project report is dedicated to The Almighty God, with whom all things are possible, and to my treasure, Ihechilurum David, Son of Excellence, Wisdom, Might and Strength, You are my Blessing.

ACKNOWLEDGEMENTS

I wish to express my profound gratitude to my supervisor, Professor C.O Nwosu for his support, encouragement, advice and for stretching himself beyond the limits of his convenience to ensure that this work turned out a success.

I am indeed also grateful to my Head of Department, Prof R.C, Ezeokonkwo, who tirelessly offered me quality academic guidance, encouragement and support. His encouragement and support were truly beneficial to the success of this work.

My weighty appreciation also goes to my teachers Prof J.I Ihedioha and Prof D.N Ezeasor for their immeasurable support, kindness, encouragement and goodwill towards me and my academic career. You spurred me on and I know the story of my success wouldn't be complete without you.

My special regards also goes to Pastor Felix Great, of Christ Embassy Nsukka, for your prayers and encouragement. I have been lifted, strengthened and carried by your Words of grace. You are a blessing to my life. God forever bless and increase you Sir.

I want to specially appreciate my siblings Amara and Ik, darlings forever, and my Mummy, extraordinary woman of strength, virtue and grace. You all have been my pillar of love and support. It would have been practically impossible for me to get here without you all. This success is our success.

To my darling son Ihechilurum David, You truly are my blessing. A true herald of your generation, an ambassador of love and divine might, the light of God shines through your heart to me.

I also want to expressly thank the following friends and colleagues for assisting and supporting me during the course of this work, Dr (s) Terry Ezeudu, Susan Dan-Jumbo, Eke

I.G, Ikenna Eze, Chijioke Iheagwam, Idika I.K, Okoye Chidozie , Ukamaka Eze, Onyeabor Amaechi, L.A Ngongeh and Mrs Justina Bassey. To my amiable Head of Department, Prof Amaechi Ndubueze, God bless you Sir and thank you for all your understanding and support. I also acknowledge the assistance of the technical and administrative staff of the Dept of Veterinary Parasitology and Entomology, UNN and other staff of the Faculty who helped me in one way or another during the course of this work. God bless you all for your support.

Finally, my greatest gratitude goes to my Father, The Author of All Life, The Head Over All Things, Eternal God and Father of My Lord Jesus Christ, in whom all fullness dwells. Thank You Lord for Your faithfulness to me and mine. Thank you for making me your glory. For all that I am, for all that I have become, I give you all the praise.

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ABSTRACT

The chemotherapy of gastrointestinal helminthosis relies mainly on the use of anthelmintics. However, concerns over drug resistance have encouraged the search for new drug leads. This project report focused on evaluating the toxicity and anthelmintic effect produced *in vitro* and *in vivo* by the methanol extract of *Duranta erecta* fruits. From the study, the plant extract was found to contain several chemical components including flavonoids, tannins, polyuronides, saponins, glycosides and terpenes. Acute toxicity evaluation of the plant extract showed that the extract had an LD₅₀ greater than 5000mg/kg BW and therefore was not acutely toxic for oral use. The *in vitro* assay showed that the plant extract had a poor anthelmintic effect (LC₅₀ Extract= 0.796mg/ml) when compared with Albendazole, a standard anthelmintic (LC₅₀ Albendazole= 0.193µg/ml). Thirty (30) male albino mice randomly distributed into six experimental groups of five animals each were used for the *in vivo* experiment. Twenty-five mice were infected with the murine nematode *Heligmosomoides bakeri* and constituted the Infected Groups (A-E) while five mice were uninfected with the parasite and served as Uninfected Control Group (F) for the experiment. Graded ascending doses (250mg/kg BW, 500mg/kg BW and 1000mg/kg BW) of the plant extract and Albendazole (25mg/kg BW) were orally administered to the mice in the infected groups respectively. Coprological and haematological parameters including Faecal Egg Counts (FEC), Packed Cell Volume (PCV), Haemoglobin Concentration (Hb), Total Red Blood Cell Counts (RBC), Total White Blood Cell Counts (WBC) were evaluated and recorded during the study period. The Body Weight (BW) and Red blood cell indices including Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were also estimated and recorded. Twenty-eight (28) days post infection, the infected mice were humanely sacrificed and the Post Mortem Adult Worm Burdens (WB) were estimated and recorded. The results showed

that the plant extract was unable to eliminate faecal egg counts or adult worms in the gastrointestinal tract of infected animals even at the high doses used, reflected by increasing FEC in treated mice throughout the study. This is in contrast to the anthelmintic effect produced by Albendazole which significantly ($p<0.05$) reduced faecal egg counts and worm burdens by 71% and 92% respectively in treated mice. There was a significant ($p<0.05$) decline in all erythrocytic parameters (PCV, HB, RBC) in all infected groups, suggestive of anaemia. Treatment with the plant extract, regardless of the dose, was unable to reverse the effect of parasite infection on erythrocytic parameters. However, treatment with Albendazole positively reversed the anemia, restoring the mice to pre-infection values by the end of the experiment. The results showed a significant ($p<0.05$) increase in White blood cell counts following infection with the parasite. Treatment with the plant extract and Albendazole significantly ($p<0.05$) reduced the WBC counts to pre-infection values in almost all treatment groups. There were no significant ($p<0.05$) decreases in the body weights of the mice post infection and post treatment with the plant extracts. This is in contrast to the decrease in body weight observed in mice in the Untreated Control group. As a result of the poor anthelmintic effects recorded in the study, it was therefore recommended that the methanol extract of *Duranta erecta* fruits be explored for its other useful effects rather than as an anthelmintic.

CHAPTER ONE

INTRODUCTION

1.1. INTRODUCTION

Helminth infections and associated diseases constitute a severe helminth problem and the single most important threat to economic success in livestock production (Perry *et al.*, 2002). The occurrence of helminth infections is usually reflected as decreased feed efficiency and productivity, poor performance, high morbidity and ultimately mortality in affected flocks (Elsa *et al.*, 2012). The treatment of gastrointestinal helminth infections has been achieved largely through the use of synthetic anthelmintics (Kaplan, 2004). Anthelmintics are drugs that act locally to expel worms from the gastrointestinal tract or systemically to eradicate adult helminths or their developmental stages which invade organs and tissues of the host (Danquah *et al.*, 2012). There are three main classes of anthelmintics currently in use for the management of helminth infections and these include the Benzimidazoles, Nicotinic acetylcholine agonists and the Macrocyclic lactones (Suarez and Cristel, 2007). A growing problem with the continued use of anthelmintics in therapy of helminth infections is the development of resistance to available anthelmintics by some of these parasites particularly in species affecting sheep, horses, cattle and pigs (Little *et al.*, 2003; Behnke *et al.*, 2008). Other challenges to its continued use include the problems of drug residues and the high cost of conventional anthelmintics (Nalule *et al.*, 2011).

Medicinal plants and fruits have been used locally by indigenous peoples for centuries in the treatment of a variety of problems, including infectious diseases and those caused by parasites like helminthes both in livestock and humans. Various scientific discoveries have shown that plants produce a wide range of complex compounds many of which have been documented to have significant anthelmintic properties (Oseni *et al.*, 2012). These medicinal

plants have been shown to have such advantages as minimal side effects, cheaper cost and no drug resistance over synthetic drugs (Pawan, 2009). These plants also tend towards providing viable alternatives to the use of synthetic anthelmintics which will help to curb an over reliance on available drugs. This will in turn help to improve efficacy and reduce the development of parasite resistance to available anthelmintics (Hammond *et al.*, 1997; Waller *et al.*, 2001).

Duranta erecta commonly called 'Golden dew drops' or 'Pigeon berry' in the United States of America or 'Yellow bush' locally here in Nigeria, is a small evergreen shrub which has characteristic bright yellow spherical fruits of about half inch in diameter and belongs to the family *Verbenacea* (Watkins *et al.*, 2005). The plant is usually used by landscapers and horticulturists in making hedges. Its bright lavender flowers and attractive golden fruit add a splash of colour to landscapes (Rushing, 2005). The plant is not browsed by cattle but birds easily feed off the fruits (Nikkon *et al.*, 2008). The fruit is also reported to be poisonous to humans (Andreu *et al.*, 2010). The ethyl acetate and aqueous extracts of the leaves have been reported to show significant anti-malarial activity when administered to mice and have also been used in the treatment of abscesses (Xiao, 1992; Castro *et al.*, 1996). The fruits have also been used in the treatment of malaria and intestinal worms (Whistler, 2000; Nikkon *et al.*, 2008). The plant is also reported to produce a wide range of steroids, flavonoids, glycosides and terpenoids (Takeda *et al.*, 1995; Anis *et al.*, 2001). Despite various reports revealing interesting results on the traditional uses of the fruit of *Duranta erecta* as an anti-parasitic agent, there is a dearth of information in available scientific literature on its toxicity and effect on gastrointestinal helminth infections or as a possible alternative in the therapy of drug-resistant gastrointestinal helminths. This project is therefore designed to evaluate the toxicity and anthelmintic properties of the methanol extract of *D. erecta* fruit so as to authenticate the basis for its folkloric uses as vermifuge.

1.2. STATEMENT OF THE PROBLEM

- Gastrointestinal parasites have become more difficult to manage because of the increasing development of resistance by these parasites to available synthetic drugs.
- There is a need to find alternatives to the presently available anthelmintics, which will help to curb an over reliance on these drugs.
- Though there are reports of traditional and folkloric use of *Duranta erecta* fruit as an anti-parasitic agent, there are no scientific reports in available literature of its use in the control of gastrointestinal helminth infections or its toxicity in treated animals.

1.3. OBJECTIVES

The overall aim of this study was to evaluate the toxicity and anthelmintic effect of the methanol extract of *Duranta erecta* fruits in mice experimentally infected with the nematode *Heligmosomoides bakeri*.

The specific objectives of the study included to evaluate;

- The phytochemical constituents of the methanol extract of *Duranta erecta* fruit
- The toxicity of the methanol extracts of *D. erecta* fruit in albino mice.
- The *in vitro* effects of the methanol extracts of *D. erecta* fruit on the eggs of *Heligmosomoides bakeri* using egg hatch assay method.
- The *in vivo* anthelmintic effects of the methanol extracts of *D. erecta* fruit in albino mice experimentally infected with *Heligmosomoides bakeri*.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

A parasite is an organism that lives in (endoparasite) or on (ectoparasite) another animal called the host. Parasites occur virtually in or on all living things and in natural conditions generally existing in comparative harmony with their hosts, outbreaks of clinical parasitism being relatively rare (Brunsdon *et al.*, 1975). Domesticated animals harbor a wide range of parasites, especially internal parasites mainly because they are usually kept in large flocks or herds and are frequently concentrated in confined areas that favour the buildup of parasite infections. Such parasite infections in domestic animals frequently result in either subclinical or clinical disease (Familton and McAnulty, 1997).

Helminth parasites are by far the most frequent causes of production losses in farm animals and nematodes are the most important among them (Chiejina, 2001). Internal parasitism is the major disease syndrome in pastoral animals because environments that favour pastoralism are the same as those which favour the development and survival of the free-living stages of helminth parasites (Sykes, 1994; Vlassoff *et al.*, 2001). The effects of helminth parasite infections vary between wide extremes. At one end, death and total economic loss can occur and at the other, the effects may be undetectable hence control measures unwarranted (Pomroy, 1997). Generally, clinical and even subclinical helminth parasite infections have economic impact on farming operations (Waller, 1997). The economic consequences of helminth infections include increased mortality, reduction in live weight gain, reduction in wool growth and quality, decreased fertility and milk production, rejection of carcasses and organs for human consumption and predisposition to other diseases (Ajayi *et al.*, 2008). Despite the immense progress made to control helminth infections,

farmers continue to incur significant losses due to insufficient availability of information on the epidemiology and control of these parasites.

2.2 HELMINTH PARASITES OF DOMESTIC ANIMALS

The helminths of veterinary importance are commonly found in two major Phyla namely: *Nemathyhelminthes* or round worms and *Platyhelminthes* of flat worms.

2.2.1 *Nematyhelminthes*

The phylum *Nematyhelminthes* contains three major classes including;

- *Nematoda* or round worms
- *Acanthocephala* or thorny-headed worms
- *Annelida* or segmented worms

The Class *Nematoda* commonly called roundworms are generally unsegmented worms which are cylindrical in shape (Urquhart *et al.*, 1996). Helminth parasites in this class include many worms of major medical/veterinary importance. They contain two major subgroups; the *Phasmida* and the *Aphasmida*, depending on the presence or absence of phasmids which are unicellular glands that open outwards and function as olfactory organs. Most nematodes have a cylindrical form, tapering at either end with their bodies covered by a colourless, translucent layer called the cuticle. The life cycle of nematodes could either be direct or indirect. Some parasitic nematodes of veterinary importance affecting different animal species include members of the following genera: *Haemonchus*, *Cooperia*, *Trichostrongylus*, *Ostertagia/Teladorsagia* and a host of others responsible for various separate diseases that are contributory to a clinical syndrome described as parasitic gastroenteritis (PGE) in ruminants; *Stephanurus* popularly known as the “Kidney worm” are found in the kidney and peri-renal tissues of pigs; *Heligmosomoides* commonly found in the small intestine of wood

mice and other rodents; *Strongylus* and *Oxyuris* found in the blood vessels and large intestine of horses; *Strongyloides* found in the small intestine of most animals and in the caecum of poultry; *Syngamus* found in the trachea of chicken, turkey and guinea fowl; *Ancylostoma*, *Ascaris*, *Toxocara* and *Spirocerca* found in the small intestine of dogs and cats; *Metastrongylus* found in small bronchi and bronchioles of pigs and *Macracanthorhynchus* in pigs with various dung beetles as intermediate host (Lapage, 1962; Amin, 1988; Soulsby, 1982).

The *Acanthocephala*, generally referred to as 'thorny headed' worms due to the presence of an eversible proboscis have their heads covered with spines and are not of much veterinary or medical importance. On the other hand, the annelids or segmented worms have a segmented body and are usually of no medical or veterinary importance except for *Hirudinea* or leaches and *Oligochaeta* or earthworms. Both groups have a clitellum or a swelling near the head that contains gonads. They are also called *Clitellata*.

2.2.2 Platyhelminthes

This phylum contains two classes of parasitic flatworms: *Trematoda* (trematodes or flukes) and *Cestoda* (cestodes or tapeworms).

The Class Trematoda

This class of flatworms has two main sub-classes namely; *Monogenea* and *Digenea*. *Monogenea* have direct life cycles and occur mainly as external parasites of fish. The *digenea* have indirect life cycle which require an intermediate host for the completion of their life cycles. They occur exclusively in vertebrate hosts.

Adult flukes occur primarily in the bile ducts, alimentary canal and vascular system of their hosts (Duncan *et al.*, 1996). Trematodes of veterinary importance include species of the

following genera: *Fasciola* found in the liver and bile ducts of ruminants and pigs; *Paramphistomum*, *Schistosoma* and *Dicrocoelium* which affect a range of animal hosts including ruminants and rabbits and *Paragonimus* found in the lungs of dogs, cats and other carnivores (Urquhart *et al.*, 1996).

The Class Cestoda

Cestodes differ from trematodes in that they have tape-like bodies with no alimentary canal. Their bodies are segmented and each segment contains one or sometimes two sets of male and female reproductive organs. The life cycle is usually indirect with one intermediate host (Soulsby, 1982). Some cestodes of veterinary importance include: *Taenia* which is an important genera of public health significance and whose adults occur in the intestine of man, dogs and cats while the larvae are found in the muscles, liver, peritoneum and central nervous system of cattle, sheep, pigs, man and rodents; *Dipylidium* and *Echinococcus* that occur in dogs and cats, and *Moniezia* found in the small intestine of ruminants.

2.3 EPIDEMIOLOGY OF NEMATODE PARASITES OF DOMESTIC ANIMALS

2.3.1 MODE OF TRANSMISSION OF INFECTION

The life cycle of nematode parasites involves a free living or pre-parasitic phase and a parasitic phase of development. In the case of strongylid nematodes, the preparasitic phase of the life cycle includes the egg that is usually passed out in the faeces of the host and which, under suitable environmental conditions, develops to a pre-hatch stage containing a fully formed embryo (Chiejina, 1986). The process of hatching releases the first stage larva (the L₁) into the environment. When the environmental conditions are favourable (optimum conditions of temperature, moisture and oxygen), the L₁ moults to the second stage larva, L₂ and finally to the third stage larva L₃ which has a double protective sheath. The L₃ is the infective stage of the parasite. The preparasitic stages including the egg, L₁, L₂ and L₃ are

usually less susceptible to adverse environmental conditions than the other developmental stages in the parasitic phase of the life cycle (L₄ and L₅) which are found within the host animal (Hansen and Perry, 1994).

There are slight modifications of this trend with respect to individual nematode parasites. For instance, larval development in the genus *Trichuris* and *Toxocara* or *Ascaris* proceed inside the egg until the L₂ and L₃ respectively are formed. Hatching of these infective eggs takes place only after ingestion by the host animal. *Strongyloides papillosus* has two life cycle types i homogonic and heterogonic cycles used respectively during favourable and unfavourable environmental conditions. This species lays eggs that already contain L₁ when passed out in the faeces of the host. Further development of the embryonated egg after the hatching of the L₁ proceeds as already described for strongylids in the homogonic cycle when conditions are favourable i.e. the parasitic life cycle stages. When environmental conditions are unfavourable, the heterogonic cycle predominates and here the L₁ gives rise directly to sexually mature free living male and female adults. Following copulation, the adults produce fully embryonated eggs which hatch after a few hours to the L₁ stage that would develop to the L₃ stage (Soulsby, 1982). The L₃ which result from both the homogonic and heterogonic cycles are the infective stages and are devoid of a protective sheath. They are therefore relatively more vulnerable to desiccation and other adverse environmental conditions than the L₃ of strongylid nematodes.

Infection of the host with strongylid nematodes occurs usually through the ingestion of the L₃ during normal grazing or feeding. Therefore the L₃ has to migrate out of faecal deposits onto grass blades in order to facilitate the process. Some other species like the strongyles of horses actively penetrate the skin of the host to initiate infection (Barger, 1999).

2.3.2 PREVALENCE OF NEMATODE INFECTIONS AND DISEASE IN GRAZING ANIMALS

Nematode infection is a common occurrence in small ruminants but the intensity of the infection as well as the clinical signs associated with the disease can vary considerably (Donald *et al.*, 1978). This is because the severity of the disease is mainly influenced by several factors that include the variety, species and the number of worms present in the gastrointestinal tract, the general health and immunological state of the host animal, and environmental factors such as climate and pasture type, stress, stocking rate, management and/or diet (Kassai, 1999).

Ruminant livestock play an important role in the socio-cultural life of farming communities as a partial determinant of wealth, and also having value as important readily convertible assets and in food production systems of developing countries (Blackburn *et al.*, 1984; Getachew, 1988). Several studies have been conducted on the prevalence of gastrointestinal nematodes affecting different animal species all over the world. In the tropics, strongyles have been shown to be the most common, economically important gastrointestinal nematodes affecting grazing animals world wide (Sykes, 1994). High prevalence rates of various gastrointestinal nematodes have been reported by several workers. In Kenya for instance, the widely reported Strongyle genera of importance include *Haemonchus*, *Trichostrongylus*, *Cooperia* and *Oesophagostomum*. (Ng'ang'a *et al.*, 2004). This is also similar to the report by Adejinmi *et al.* (2015) in South West Nigeria where a total prevalence rate of 75.75% was observed in Red Sokoto goats. It was suggested in the study that the observed high prevalence rate may have occurred due to the management system of the animals. Prevalence rates of 76.5% and 35% have also been reported in South West Nigeria and North Eastern Nigeria respectively (Sylvia *et al.*, 2015). *Haemonchus contortus*, *Trichostrongylus axei*, *Nematodirus axei*, *Teladorsagia circumcincta* and *Cooperia* are the

most implicated parasite species causing disease and economic losses in grazing animals. However, *Haemonchus contortus* is considered the most pathogenic of the species affecting ruminants (Perry *et al.*, 2002; Waller *et al.*, 2001).

Usually, three main groups of animals are more prone to heavy worm burdens; (i) young, non-immune animals, (ii) adult, immune-compromised animals, and (iii) those animals which have been exposed to high infection pressure from the L₃ contaminated environment (Elsa *et al.*, 2012). Nematode populations in grazing small ruminants (pastured) generally are over-dispersed, with only few animals carrying heavy worm burdens, whilst the majority of the animals harbor low numbers of worms. Estimation of pasture burden of infection may therefore wrongly suggest high infection rates. However, direct faecal examination and counts from suspect animals in an environment more reliably reflect the endemicity of a parasite in the environment. Also for strongylid nematodes, faecal culture and harvesting of larval stages more accurately represents the prevalence of the parasite since most of the parasites may not be readily differentiated at their egg stages. This is important because different nematode parasites have varied fecundity and a high faecal egg count may wrongly suggest a high number of adult parasites present in the host animal.

2.3.3 FACTORS AFFECTING THE SIZE OF GASTROINTESTINAL NEMATODE INFECTIONS

According to Hansen and Perry (1994), there are five main factors which influence the size of any nematode infection and they include:

1. The number of L₃ which is ingested by the host. This is in turn influenced by the climate, the amount of the protection of the larvae provided by vegetation, livestock density and the grazing pattern of ruminants present.

2. The intrinsic rate of multiplication of the parasite species present which is controlled by pre-patent period, fecundity and survival rate of the parasite.
3. The rate at which acquired resistance develops in the host, which is influenced by the species of the parasite and host, genetic factors, nutrition and physiological stress such as parturition.
4. Management, especially grazing patterns.
5. Use of anthelmintics, including timing and frequency of administration

2.4 PATHOGENIC EFFECTS AND CLINICAL SIGNS OF HELMINTHOSIS

The pathogenic effects of helminth parasites may be sub-clinical or clinical. Young animals are most susceptible. The effect of these parasites is dependent on the number of parasites and the nutritional status of the host animals. Also, clinical signs such as weight loss, reduced feed intake, diarrhea, reduced carcass quality, reduced wool production and wool quality and mortality could be seen in affected animals (van Wyk, 1990; Ngongeh *et al.*, 2007). Severe blood and protein loss into the abomasums and intestine due to damage caused by adult parasites often results in oedema in the submandibular areas and anaemia (Morley and Donald, 1980). Anaemia is a major pathogenic effect produced by most gastrointestinal nematode parasites in their host. This is essentially haemorrhagic anaemia due to the blood sucking habits of the worms. Thus, each haematophagous worm can remove up to 0.05ml of blood per day by ingestion and seepage from the lesions it produces in the mucosa of the host (Wabo *et al.*, 2010). This is largely due to high fecundity of adult worms producing large numbers of eggs and infective stages which could accumulate in high numbers in pasture within a short period of time. The most obvious forms of direct damage caused by adult parasites are those resulting from the blockage of internal organs or from the effects of pressure exerted by growing parasites (Stear *et al.*, 2003). The larval stages of parasitic

nematodes usually cause considerable damage in the host, especially the fourth stage larva L₄ of abomasal parasites. The L₃ enters the mucous membranes or the glands in the wall of the abomasum within six hours of infection. They usually stay within the mucous membrane or the abomasal glands for about two to three weeks. Thereafter, clinical signs such as reduced appetite, reduced digestive capability of the abomasum will usually result (FAO, 2005). The L₄ of *Haemonchus contortus* is a blood sucker. Animals affected with large numbers may therefore suffer from anaemia before the parasite eggs may be detected in the faeces of the host animal (Brunsdon, 1980; Wakelin, 1996). Some parasites produce spoliative effects in their hosts as they deprive the host of their food when located in privileged sites within the host animal. They absorb already digested food materials necessary for the host's normal functions. Implicated parasites include *Trichostrongylus*, *Haemonchus* etc. In some cases, large numbers of parasites migrate within host's tissues leading to trauma which can lead to shock or rupture of the tissues e.g damage to liver of pigs caused by *Ascaris*. Clinical signs such as inappetence or anorexia are common with infection of animals with some nematode parasites including *Oesophagostomum*, *Cooperia*, *Trichostrongylus* etc. this usually occurs by interference with haemopoiesis in the host animal. Several nematodes may also cause host reactions such as inflammation, hypertrophy, hyperplasia and nodule formation in their host's tissues. Repeated contact between the host and the parasite sensitizes the host to the antigens of the parasite and may produce varied physiologic and pathological effects.

2.5 DIAGNOSIS OF HELMINTHOSIS

Diagnosis of helminthiasis is mainly by;

- a) History
- b) Clinical signs
- c) Gross and histopathology examinations

d) The use of Laboratory aids.

History involves investigations into the prevalence and occurrence of suspected parasite populations in the area, management practices including stocking density, hygiene and nutrition, physiologic status of affected animal etc. Clinical diagnosis involves physical examination of the animal for signs associated with helminth disease or pathological conditions produced by parasite infection (WHO,1991). Laboratory aids include direct faecal examination, qualitative examination of faecal samples for the presence of helminth egg by techniques such as direct wet mount, saline flotation or sedimentation techniques which indicate the presence or absence of helminth eggs in such samples. There are also quantitative methods of faecal examination which estimate the number of parasite stages present in specific quantities of collected faecal samples. Examples of such techniques include the McMaster egg counting technique. Post mortem parasite burden estimation can also be carried out in diagnosis of helminth infections (Roepstorff and Nansen, 1998).

Direct faecal examination is commonly used in the diagnosis of helminth infections especially in field cases. This involves the observation of the consistency, colour, and odour of collected faecal samples. Wet mounting is the simplest and easiest laboratory technique for faecal examination. This can be prepared directly from faecal materials or from concentrated faecal specimens. There are two main types of wet mounts which can be used for faecal examination for helminth parasites and these include;

1. Saline wet mount
2. Iodine wet mount

In the direct saline and iodine mounts, a drop of saline or iodine is placed on a microscope slide. With an applicator stick, a small portion of faecal specimen is picked up and mixed

with the saline or iodine respectively. The mixture is then covered with a coverslip and viewed under the low power objective of a light microscope to determine the presence or absence of parasite stages (WHO, 1991).

2.5.1 FAECAL EXAMINATION AND EGG COUNTS

The most commonly used laboratory method for the qualitative diagnosis of gastrointestinal parasites involves the simple test tube floatation test methods used to examine faecal samples in order to detect helminth eggs (Urquhart, 1987). Faecal egg count, faecal culture and harvesting of infective larvae and adult worm recovery and count at necropsy are also commonly used laboratory diagnostic methods.

Faecal egg count is the most common ante ï mortem method of diagnosis of helminthiasis and is based on the assumption that the adult worm burden in animals may be accurately deduced from the number of parasite eggs found in the faeces of the host. The technique is cheap and relatively easy to perform. The McMaster technique is a rapid, easy and most commonly used method in quantitative analysis of nematode eggs in the field and in epidemiological surveys (Hansen and Perry, 1994).

2.5.2 POST MORTEM WORM COUNTS

Post mortem parasite counts provide a more precise assessment of parasite burdens than parasite egg counts. For post mortem worm counts, the intestinal tract from abomasums to the rectum is usually required. The adult and larval nematodes are carefully washed out, counted and identified. It is important to record all abnormalities and lesions observed. This technique permits identification of adult and or developing worm stages present and a direct count of the number of worms present in animals post mortem, thereby providing a precise assessment of the worm burden. It is considered to be the most accurate method of diagnosis of

helminthosis. However, it is expensive to perform because it requires the use of whole animals post mortem (Roepstorff and Nansen, 1998).

2.6 PREVENTION, CONTROL AND TREATMENT OF HELMINTHOSIS

Helminthosis is a major constraint to livestock productivity hence control of these parasites is necessary. The general aims of nematode control are to limit host challenge to a level which does not compromise performance or welfare while at the same time enabling the development of immunity. Control may be based on grazing management, prophylactic anthelmintic treatment and integrated parasite management systems taking into account these two management schemes (Probert, 2001).

2.6.1 CONTROL BY GRAZING MANAGEMENT

This method aims at reducing the contact of susceptible animals with infective stages of helminth parasites. This control method is considered to be more sustainable than the use of anthelmintics and may be the most practical method for use by farmers who cannot afford to purchase expensive drugs. This method incorporates the knowledge of the epidemiology of the helminth parasite and other management practices like good hygiene and adequate nutrition. Some of these strategies include;

- i. **Reduction of Stocking Densities:** Overcrowding of animals in an area could lead to high rate of pasture contamination resulting in high level of infection of susceptible animals. Therefore farmers should encourage adequate stocking densities and prevent overcrowding (Torres-Acosta and Hoste, 2008).

- ii. **Adequate Nutrition and Hygiene:** Clean housing area reduces parasite burdens and consequently reduces infection rates. Well nourished animals are also better able to tolerate the presence of or resist the establishment of parasites (Barger, 1999).
- iii. **Timing of Reproductive Events:** This is equally an important helminth control strategy. Reproductive events such as lactation and pregnancy (especially late pregnancy) are usually associated with immunosuppressive effects in host animals. Consequently, arrested larvae present in host animals emerge and re-continue their development in such situations leading to a periparturient rise in faecal egg counts and high pasture contamination with infective larval stages. Strategic deworming of animals at this stage will eliminate adult worms, reduce pasture contamination with infective larvae and prevent infection of susceptible young animals in a herd (van Wyk, 1990).
- iv. **Alternative Grazing with Animals of a Different Species:** Helminth parasites are usually host specific hence alternate grazing of pasture with hosts of different species from time to time could help reduce pasture parasite burden. For example grazing a pasture with horses followed by ruminants would help reduce pasture burdens of ruminant parasites. The horses would take up most of the pre-parasitic stages of the ruminant parasites available on the pasture. These pre-parasitic stages of ruminant parasites, on the other hand, would not be able develop further in the equine host such that the infection would be abrogated (Niezen *et al.*, 1996).
- v. **Pasture resting:** Here, animals are withheld from designated pasture for a period of at least 6 months. This would enable reduction of pre-parasitic larvae concentration to the barest minimum. Most pre-parasitic stages of helminth parasites would not be able to survive in the environment without the final host within such a period leading to mortality of these stages and consequent reduction of pasture parasite burdens within

this period. Re-introduction of animals to such pastures after the rest period will therefore limit infection rates. (Brunsdon, 1980; Cabaret *et al.*, 2001).

- vi. **Rotational grazing:** This involves dividing the pasture land into parcels of land of varying sizes called paddocks. Animals are intermittently moved between paddocks and this ensures reduction of infection risk till larval concentration is significantly diminished in already grazed paddocks such that by the time animals are returned to a certain paddock, there would be maximum reduction of pasture burdens of infective stages (Barger, 1996).

2.6.2 CONTROL BY CHEMOPROPHYLAXIS

This method aims at eliminating the parasitic stages of the parasite in the host, thus preventing the passage of eggs and larvae into the environment. Chemoprophylaxis is usually aimed at pre-empting the occurrence of clinical disease caused by parasites in the host. This is different from Chemotherapy which is carried out after the animal has succumbed to clinical disease. Chemoprophylaxis is the most commonly and extensively used method for the control of helminthosis throughout the world (Kaplan, 2004). It is therefore recommended that the frequency of treatment should be determined by the epidemiology of the dominant parasite, the stocking rate in the particular area and the benefits accrued from the control regime adopted (Sangster, 1999). This is particularly necessary as a result of the emergence of resistant strains of worms. In humid climates in Nigeria for instance, the environmental conditions are permanently favourable for the development, survival and transmission of infective larvae. In these climates, it is important to ascertain levels of parasitism and the epidemiology of the species present in order to satisfactorily determine the frequency and timing of strategic anthelmintic dosing.

Frequent and indiscriminate use of anthelmintics interferes with the development of immunity against helminthes by animals and this could lead to selection of resistant genes (Prichard, 1990). This has become a serious problem in many countries and resistance of parasites to one or more anthelmintics is now widespread, particularly in sheep. The loss of inexpensive drugs against helminthes as a result of development of anthelmintic resistance has made it expedient to explore and develop new ways of worm management in order to reduce selection for resistance. There is also increased need for alternatives to the use of anthelmintics for parasite control (Waller, 1997).

2.6.3 TREATMENT

Treatment of helminth infections is usually by use of anthelmintics. These are drugs that act either to eradicate adult or developmental stages of helminthes that invade organs and tissues of the host (Theiler, 1912). An ideal anthelmintic should be broad spectrum in action; acting against mature and immature parasites including hypobiotic larvae. It should be safe, easily administered, rapidly metabolized and excreted and compatible with other compounds (Danquah *et al.*, 2012). There are three major classes of broad spectrum anthelmintics in current use and they include Benzimidazoles and Imidazothiazoles, Hydropyrimidines and the Macrocyclic Lactones. Treatment with anthelmintics is usually carried out when the affected animals are already showing obvious clinical signs of infection by the parasite.

The timing of anthelmintic administration is an important consideration as a result of the phenomenon of hypobiosis. Here, developmental stages of parasites trapped or embedded in the mucosa of the gastrointestinal tract of their host and remain largely inactive and relatively harmless to the host owing to unfavourable environmental conditions. They resume their development when environmental conditions become favourable for the development of

their pre-parasitic stages or when the immune system of the host animal becomes compromised as occurs during concurrent infections or during some physiological processes like pregnancy. Treatment with anthelmintics at this stage shows no significant effect. Hence, administration of anthelmintics should be timed to correspond with the emergence of these hypobiotic larvae in their hosts which would usually produce clinical disease in the affected host animal.

There are several limitations to the use of anthelmintics as major means of controlling helminth infections and these include:

- i. **Development of Resistance:** The regular use of anthelmintics as parasite control strategy has led to the selection of drug resistant helminthes. The problem of resistance to anthelmintics has led to the loss of inexpensive drugs against helminthes and hence a major problem for the control of helminth parasites (Conder and Campbell, 1995; Idika *et al.*, 2012).
- ii. **Under-dosing:** Most farmers under dose their animals by underestimating the weight of their animals. The case is further complicated when manufacturers recommend doses for a broad weight range. Also, there are differences with regards to metabolism of drugs between the two small ruminant species (Sheep and goats). Most farmers do not consider these differences and administer generalized doses to their animals, leading to possible under-dosing or over-dosing of affected animals (Martin, 1989; Hoekstra *et al.*, 1997).
- iii. **Rapid Rate of Re-infection:** If animals are grazed on heavily contaminated pastures, re-infection occurs frequently and this may give the impression of drug failure (Coles *et al.*, 1995).

- iv. **Introduction of Resistant Parasites** by means of animals transported from country to country or from farm to farm. Consequently, the drugs in use in an area are unable to eliminate parasite burdens in affected animals because of the presence of resistance genes to available anthelmintics in the implicated parasite species. (Little *et al.*, 2003).

Considering these challenges to the continued use of synthetic drugs as therapeutic agents to combat the problem of helminth infections, there is a great need for newer, more reliable, efficacious, commonly available and environmentally friendlier anthelmintic agents. Herbal medicines present a suitable alternative in this case.

2.7 ETHNOVETERINARY MEDICINE

Ethnoveterinary medicine (EVM) refers to people's knowledge, skills, methods, practices and their beliefs about the care (treatment) of their animals (McCorkle, 1986). Ethnoveterinary medicine considers that traditional practices of veterinary medicine are legitimate and seek to validate them (Kohler-Rollefson, 1994). Stock owners continue to utilize EVM until better alternatives in terms of efficacy; low cost, availability and ease of administration are found. The most studied element of EVM is Veterinary Ethnopharmacopoeia, especially plants (Warren, 1991).

2.7.1 POTENTIALS OF PLANTS AS ANTHELMINTHICS

Plants are natural products which have shown an advantage of production of less harmful effects and with less or almost no deposition of residues in the treated individual. They have also been shown to regulate life processes and prevent diseases (Egualé *et al.*, 2011). Several plants have been shown traditionally to have medicinal value in the control of helminthosis and other ailments in man and animals. Many antibiotics, anthelmintics, anti-oncogenic,

antiprotozoan and a host of other drugs have been produced from plants (Onyeyili *et al.*, 2001). Plants also provide cheaper alternatives to western (synthetic) drugs. It has also been suggested that the diversity of herbal anthelmintics has precluded the occurrence of resistance despite several years of use (Nwude and Ibrahim, 1980). Herbal remedies could also generate useful information needed in the development of drug alternatives for livestock which are suited to the local environment. It also presents a key veterinary resource and has the potential to add new drugs to the Pharmacopoeia (Cotton, 1996).

2.7.2 CHALLENGES TO THE USE OF PLANTS AS ANTHELMINTHICS

A major challenge of EVM is that it does not follow western paradigms of scientific proof of efficacy and this forms the basis of the major argument against the use of traditional medicine today (Sofowora, 1982). Also significant are the seasonal availability of certain plants and the inconvenience of preparing or using some remedies. Collection and preparation of some of these plant remedies can be very time consuming. Also, each constituent may have to be found only in special habitats (Schillhorn van Veen, 1997). There is also the challenge of scarcity of herbal treatment following the occurrence of infectious epidemic diseases and often inadequate ethnodagnosis owing to lack of pathophysiological understanding of disease processes, culminating in inappropriate or harmful prescription (Bizimana, 1994).

2.7.3 SOME NATURAL PRODUCTS WITH ANTHELMINTHIC PROPERTIES

Several plants have been shown to have anthelmintic properties and they include leaves of *Acalypha fimbriata*, *Clerodendrum paniclatum*, *Delonix regia*, *Greenwagodendron spp*, *Lageneria sicerata* and *Neruyum oleander* (Odegbemi, 2006). Dried seeds of *Abrus prectorius* (Ibrahim, 1992), *Acacia cookii* dried bark (Caceres *et al.*, 1990), *Aegle marmelos* seed oil (Singh *et al.*, 1983), *Agapetes serpens* entire dried plant (Aswal *et al.*, 1984), *Albizia*

anthelmintica bark (Galal *et al.*, 1991), *Allium sativum* dried and fresh bulb (Pena *et al.*, 1988; Middleton and Tatlock, 1998), *Aristolochia bracteolata* dried leaf (Rao, 1980), *Artemisia sivesiana* dried entire plant (Prakash *et al.*, 1980), *Balanites aegyptiaca* dried kernel (Ibrahim, 1992). *Mangifera indica* (kernel), dried leaves and bark of *Melia azedarach* (Kightlinger *et al.*, 1996), dried fruit of *Myrsine africana* (Kakrani and Kalyani, 1983) were all found to possess anthelmintic activities.

2.8 BOTANICAL PROFILE, CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF THE PLANT, *Duranta erecta*

The plant, *Duranta erecta* is commonly known as ‘Golden Dew Drops’ or ‘Pigeon berry’ in the United States of America or ‘Yellow bush’ in Nigeria and belongs to the family Verbenaceae. It usually grows as a shrub, herb or small tree frequently 1 to 3 metres in height and is also grown as a common hedge plant around human residencies and other environments (David, 1981). It is a small evergreen shrub which has characteristic bright yellow spherical fruits of about half an inch in diameter (Watkins *et al.*, 2005). The plant is usually used by landscapers and horticulturists for making hedges. Its bright lavender flowers and attractive golden fruit add a splash of colour to landscapes (Rushing, 2005). The plant is not browsed by cattle but birds easily feed off the fruits (Nikkon *et al.*, 2008). The fruits are reported to be poisonous to humans when consumed in their raw state but however have been reported to be used in the treatment of malaria and intestinal worms (Whistler, 2000; Nikkon *et al.*, 2008; Andreu *et al.*, 2010). The ethyl acetate and aqueous extracts of the leaves have been reported to show significant anti-malarial activity when administered to mice and have also been used in the treatment of abscesses (Xiao, 1992; Castro *et al.*, 1996). The plant has been reported to contain a wide range of steroids, flavonoids, glycosides and terpenoids

(Takeda *et al*, 1995; Anis *et al.*, 2001) which are possible components for anthelmintic activity.

CHAPTER THREE

MATERIALS AND METHODS

3.1 COLLECTION AND PROCESSING OF EXPERIMENTAL MATERIALS

3.1.1 Collection and Processing of Experimental Parasite Stages

The parasite, *Heligmosomoides bakeri* was used in this study. The infective stages of the parasite were kindly supplied by Dr. Lucas Ngongeh of the Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. The parasites were multiplied in donor mice at the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka.

Freshly voided faeces from the donor mice were obtained by placing the mice in clean plastic cages for 10 to 20 minutes. The voided faeces were collected in labeled containers, crushed and washed through a tea strainer with saturated sodium chloride (NaCl) solution (MAFF, 1977). The mixture was poured into a test tube and placed on the bench to allow the eggs to float to the top of the tube. After about 15 minutes, the top portion of the fluid containing the eggs was poured into another tube which was then filled with water to dilute and wash out the salt solution. The tube containing the eggs was centrifuged at 2000 revolutions per minute (-g) for 3 minutes after which the supernatant was decanted. The sediment containing the eggs was then reconstituted with more water and centrifuged again as described above. This procedure was repeated three times after which the sediment containing the 'cleaned' eggs was collected into a universal bottle and the number of eggs in 1 ml suspension was determined by serial dilution method. One milliliter of the egg suspension was further diluted

to contain approximately 20 eggs in 10 µL to be used for the egg hatch assay (Urquhart *et al.*, 1987). The stock solution was stored in a refrigerator at -4°C.

3.1.2 Collection and Extraction of Plant Material

Fresh fruits of *Duranta erecta* used in this study were collected from the environment of the University of Nigeria, Nsukka, Enugu State, Nigeria in the early rainy season. They were identified by Mr, Ozioko, a Plant Taxonomist with the Bioresources Development and Conservation Programme (BDGP) Nsukka, where a voucher specimen was deposited.

The plant sample was air-dried at room temperature (25°C) to avoid solar leaching and ground to powder using a Hammer-mill (®Star Trace Pvt. Ltd, Chennai) with a 2 mm filter. The powder was then stored in an airtight container at room temperature (25°C) until required for use. The dried powder was then added to a round bottom flask and exhaustively extracted with 70% methanol for 48 hours. The extraction was carried out by cold maceration at room temperature (25 to 28°C) with intermittent shaking at 2 hour intervals. The extract was then filtered using Whatman No 1 filter paper and the filtrate concentrated *in vacuo* using a hot air oven at 40°C. A rotary condenser (Quickfit, England) was used to recover the methanol from the filtrate. The extract was stored at 4°C until required for use. The percentage yield of the extract was calculated using the formula;

$$\text{Percentage yield (w/w)} = \frac{\text{Weight of the extract (g)}}{\text{Weight of Powder (g)}} \times 100$$

3.2 PHYTOCHEMICAL ANALYSIS OF EXTRACT

The methanol extract of *Duranta erecta* fruits was subjected to the various phytochemical analysis as described by Trease and Evans (1989). Two grams (2g) of the dried plant extract was dissolved in 20 ml of distilled water to form a solution.

3.2.1 Test for Carbohydrate

A few drops of Molisch's reagent were added to 1 ml of the extract in a test tube. Distilled water was used as the control. The observation or absence of a brown precipitate indicated the presence or absence of carbohydrates in the extract.

3.2.2 Test for Starch

A few drops of iodine solution were added to 2ml of the extract in a test tube. The observation of a blue-black interface would indicate the presence of starch in the extract.

3.2.3 Test for Glycosides

Equal volumes of Fehlings solution 1 and 2 were added to 2ml of the extract in a test tube. The mixture was heated in a boiling water bath. A dirty brown colour with orange to red precipitate would indicate the presence of glycosides in the extract. Distilled water was used as control.

3.2.4 Test for Saponins

3.2.4.1 Emulsifying test

The extract was dissolved in ethyl acetate in a test tube and a few drops of olive oil was added. The mixture was vigorously shaken. Distilled water was used as control. A faint brown emulsion would indicate the presence of saponin.

3.2.4.2 Frothing Test

Two milliliters of the extract solution was shaken vigorously and allowed to stand for 5 seconds. Equal volume of distilled water in another test tube was used as control. Observation of frothing would indicate the presence of saponin.

3.2.5 Test for Alkaloids

To about 2 ml of the extract in 3 different test tubes, few drops of Dragendorff's, Mayer's and Wagner's reagents were added respectively. The tubes were shaken and allowed to stand for 10 seconds and observed for colour changes and formation of precipitates. Pinkish, white and brown precipitate formation for Dragendorff's, Mayer's and Wagner's reagents respectively would indicate the presence of alkaloids.

3.2.6 Test for Tannins

Few drops of lead sub-acetate solution were added to 2 ml of the extract in a test tube. A light brown precipitate would indicate the presence of tannins. To another test tube containing about 2 ml of the extract, few drops of 20% sulphuric acid solution was added. A brownish precipitate would indicate the presence of tannins. To another test tube containing 2 ml of the extract, a few drops of 20% ferric chloride solution was added. The presence of a dirty brown precipitate would indicate the presence of tannins. Distilled water was used as a control in each test.

3.2.7 Test for Flavonoids

Five milliliters (5 ml) of distilled water and 1 ml of 20% sodium hydroxide solution were added to a test tube containing 1 ml of the extract. A distinct yellow coloration would indicate the presence of flavonoids. To 2 ml of the extract in a test tube, few drops of ammonia solution was added and shaken vigorously in order to confirm the presence of

flavonoids in the extract. Three distinct colours in the test tube; green (top), amber (middle) and colourless (bottom) would confirm the presence of flavonoids in the extract.

3.2.8 Test for Sterols And Terpenes

Two milliliters (2 ml) of the extract in a beaker was evaporated to dryness. The residue was dissolved in 1 ml Acetic Anhydride and 1 ml Chloroform. The solution was transferred to a dry test tube and 2 ml of concentrated Sulphuric acid was added to it. The formation of a brown or violet ring at the zone of contact with the supernatant would indicate the presence of sterols and terpenes.

3.2.9 Test for Polyuronides

To a test tube containing 5 ml of absolute ethanol, 2 ml of the extract was added in drops. It was observed for the formation of violet blue precipitate.

3.3 EVALUATION OF ACUTE TOXICITY OF THE EXTRACT

The acute toxicity of the extract was evaluated as described by Lorke (1983). Thirteen albino mice were used for the study. For the first experiment, nine mice were randomly selected and divided into three groups of 3 animals each. The animals in each group were fasted overnight and subsequently received graded doses; 10 mg/kg bw, 100 mg/kg bw and 1000 mg/kg bw *per os* of the plant extract respectively. The plant extract was constituted by dissolving 1g of the dried extract in 10mls of distilled water to produce a solution with a concentration of 100 mg/ml. The animals were observed for signs of toxicity and mortality for 24 hours. After the period of observation within which no signs of toxicity or mortalities were recorded among the treated groups, four individual mice were given oral doses of 1000 mg/kg bw, 1600 mg/kg bw, 2900 mg/kg bw and 5000 mg/kg bw of the plant extract respectively as recommended by Lorke (1983). They were also observed for signs of toxicity and mortality

for 24 hours and the results were recorded. The Median lethal dose (LD₅₀) of the extract was then estimated as described by Lorke (1983).

3.4 *In vitro* ANTHELMINTHIC SCREENING OF THE METHANOL EXTRACT OF *Duranta erecta* FRUIT

The anthelmintic effect of the plant extract was evaluated *in vitro* using Egg Hatch Assay.

3.4.1 Preparation of Standard Drug Dilution for *in vitro* Anthelmintic Screening

One millilitre of Shanuzole® (25 mg/ml, 2.5% w/v Albendazole) was dissolved in 1000 ml distilled water to get a stock solution (25 µg/ml Albendazole) to be used for the *in vitro* anthelmintic screening tests.

3.4.2 Preparation of Plant Extract Dilution for *in vitro* Anthelmintic Screening

One gram of the plant extract was dissolved in 10 ml distilled water to get a stock solution (100 mg/ml of extract) to be used for the *in vitro* anthelmintic screening tests.

3.4.3 Procedure for Egg Hatch Assay

The *in vitro* anthelmintic effect of the extract was evaluated using the egg hatch assay method as described in the World Association for the Advancement of Veterinary Parasitology (WAAVP) guidelines (Coles *et al.*, 1992) with minor modifications.

Stock solutions of the plant extract and Albendazole were respectively used to prepare 12 separate concentrations of each solution by two-fold serial dilution using de-ionized water as diluent. One hundred and fifty microlitres (150 µl) of each prepared concentration of the standard drug and plant extract solutions respectively was then added into 12 different experimental wells of a 96 flat bottom microtitre plate (Dynatech Immulon®). Control wells received only the diluents. Thereafter, 50µL of worm egg suspension containing

approximately 20 *Heligmosomoides bakeri* eggs were added to each well. The plates were incubated at 27°C for 48 hours after which a drop of Lugol's iodine was added to each experimental well. The number of eggs and hatched larvae in each of the experimental wells was counted. Lethal concentration (LC₅₀) of the plant extract and Albendazole respectively was calculated by log probit analysis (Finney, 1971). The experiment was performed in triplicate. The percentage inhibition of egg hatch was calculated using the formula:

$$\text{Percentage inhibition of egg hatching} = \left(\frac{a-b}{a} \right) \times 100\%$$

Where **a**= Total number of larvae and eggs in a particular well and **b**= Number of larvae in that well.

3.5 *In Vivo* ANTHELMINTHIC SCREENING OF THE METHANOL EXTRACT OF *Duranta erecta* FRUIT

3.5.1 Experimental Animals

Thirty male albino mice weighing between 25 and 30 g each were used for the study. The mice were kept in clean metal cages in the Animal House of the Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka and allowed 2 weeks to acclimatize to their new environment prior to the commencement of the experiment. The mice were fed *ad libitum* with commercially produced poultry feed (Top Feeds Limited Starter mash, 1 Eagle Flour Road, Ibadan North, Oyo State). Adequate water was also provided. The experimental animals were handled in accordance with International Guidelines for the use of Laboratory Animals for Biomedical Research (NRCNA, 2011).

3.5.2 Experimental Infection of Mice

Twenty five mice were infected orally with about 100 *H. bakeri* L₃ contained in 0.2ml of distilled water using an 18 G needle with a blunt tip and mounted on a 1 ml syringe. Between 8 to 10 days post infection, droppings from infected mice were obtained and examined qualitatively for the presence of *H.bakeri* eggs using simple salt floatation method to establish infection (Ngongeh and Fakae, 2011; Enejoh *et al.*, 2015).

3.5.3 Experimental Evaluation of the Anthelmintic Effect of the Extract

Following patency, the mice were randomly assigned to 6 groups (A to F) of 5 animals each. Graded doses of the plant extract and Albendazole were administered once orally on day 12 post infection to each mouse as follows;

- **Group A: Infected + Untreated.**
- **Group B: Infected + treated with 1000 mg/kg bw Extract.**
- **Group C: Infected + treated with 500 mg/kg bw Extract.**
- **Group D: Infected + treated with 250 mg/kg bw Extract.**
- **Group E: Infected + treated with 25 mg/kg bw Albendazole.**
- **Group F: Uninfected + Untreated.**

Body weights, faecal egg counts and haematocrit values were estimated and recorded every two days from the onset of the experiment. Haematological parameters including red blood cell (RBC) and total white blood cell (WBC) counts, haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin Concentration (MCHC) were evaluated every four days from the onset of the experiment. At the end of the experiment (28 days post infection), post mortem worm counts were estimated.

3.6. SAMPLE COLLECTION

3.6.1 Faecal Sample

Freshly voided faeces from donor mice were obtained by placing the mice in clean plastic cages for 10 to 20 minutes. The voided faeces were collected in labeled containers and taken to the laboratory for immediate examination.

3.6.2 Blood Sample

Blood samples for haematology were collected from the retrobulbar plexus of each mouse into sample bottles containing ethylene diamine tetracetic acid (EDTA) as anticoagulant. In each case, the sample bottle was gently rocked (shaken) to thoroughly mix up the blood with the EDTA to prevent clotting.

3.7 Parasitological Procedures

3.7.1 Faecal Examination and EggCount

Qualitative faecal examination was performed by the floatation technique using saturated salt (NaCl) solution as the floating medium (MAFF, 1977) pre-infection with the parasite and subsequently, every two days post infection till patency. Following patency and treatment with the plant extract and Albendazole respectively, quantitative faecal egg counts were performed every two days till the end of the experiment. Quantitative examination of faecal samples was carried out using the modified McMaster technique and saturated sodium chloride solution as the floating medium (MAFF, 1977). Faecal egg counts (FEC) expressed as number of eggs per gram (Epg) of faeces.

3.7.2 Post Mortem Worm Count

At the end of the experiment, all mice were deprived of food for 24 hours so as to empty the gastrointestinal tract (GIT) to ease the worm counting process. The mice were euthanized in a chloroform chamber and the GIT removed immediately. The adult worms were recovered and counted as described by Ngongeh and Fakae (2011).

The percentage de-parasitization by the plant extract and Albendazole was calculated using the following formula (Suleiman *et al.*, 2005):

$$\frac{N - n}{N} \times 100$$

Where; **N** = mean number of worms found in untreated control mice and **n** = mean number of worms found in treated mice. Percentage de-parasitization of 70 and above was considered significant in this experiment.

3.8 Haematology

3.8.1 Determination of Packed Cell Volume (PCV)

The PCV was determined by the microhaematocrit method (Coles, 1986). A microcapillary tube was 75% filled with blood and sealed at one end using plasticine. It was centrifuged at 10,000 -g for 5 minutes using a microhaematocrit centrifuge. After centrifugation, the PCV was determined using a microhaematocrit reader and recorded as percentages

3.8.2 Determination of Red blood cell (RBC) count

The Total red blood cell count was performed using the improved Neubauer counting chamber. Here, 0.02 ml of blood was added to 4 ml of the red blood cell diluting fluid in a clean test tube to make 1:200 dilution of the blood sample. The diluted blood sample was loaded in a Neubauer counting chamber that was placed on a microscope stage for 2 minutes

to allow the cells settle down. Thereafter, all the red blood cells in the five groups of 16 small squares in the central area of the Neubauer chamber were enumerated using a light microscope (x40 objective). The number of cells enumerated for each sample was multiplied by 10,000 to obtain the number of Red Blood Cells per microlitre of blood (Schalm *et al.*, 1975).

3.8.3 Haemoglobin Concentration

The cyanomethaemoglobin method was used to determine the Haemoglobin (Hb) concentration (Tietz, 1976). This was done by adding 20 µL of blood to 4 ml of Drabkin's solution in a test tube and thorough mixing them. The mixture was allowed to stand for 10 minutes before a portion was transferred to a cuvette and the concentration determined with a spectrophotometer at a wavelength of 540 nm. The Hb concentration was recorded.

3.8.4 Calculation of Red Blood Cell Indices

The mean corpuscular haemoglobin concentration (MCHC) which refers to the average concentration of haemoglobin in the RBCs in a sample was calculated using the following formula:

$$\text{MCHC (g/dL)} = \text{Hb (g/dL)} \times 100 / \text{haematocrit (\%)}. \quad (1)$$

The Mean Corpuscular Haemoglobin (MCH) which refers to the average weight of haemoglobin in the RBCs in a sample was calculated thus:

$$\text{MCH (pg)} = \frac{\text{Hb (g/dL)} \times 10}{\text{RBC (x } 10^{12})}. \quad (2)$$

The mean corpuscular volume (MCV) which refers to the average size of the RBCs constituting a sample was calculated by the formula;

MCV (femtolitres or 10^{-15} Litres) = Haematocrit (%) x 10 / RBC ($\times 10^{12}$ /L).

3.8.5 Total White Blood Cell Count (WBC)

The WBC count was performed using the improved Neubauer counting chamber method (Schalm *et al.*, 1975). Here, 0.02 ml of blood was pipetted into a small test tube containing 0.38 ml of white blood cell diluting fluid to make 1:20 dilution of the blood sample. The diluted sample was loaded onto the Neubauer counting chamber, allowed to stand for 2 minutes on the microscope stage and all cells on the four corner squares were counted. The total number of cells counted was multiplied by 50 to obtain the Total White Blood Cell count per microlitre of blood (Schalm *et al.*, 1975).

3.9 BOBY WEIGHT (BWT) DETERMINATION

Each mouse was weighed on day 0 and every two days from the commencement of the experiment by placing it on a weighing balance and recording the body weight in grams.

3.10 ADMINISTRATION OF EXTRACT AND ANTHELMINTHIC

Shanuzole® (Jawa International Limited, Lagos, Nigeria), a brand of Albendazole, purchased from a reputable pharmacy was used in the study. The drug and the extract were administered orally to each mouse using an 18 G needle with blunt tip and mounted on a 1ml syringe.

3.11 STATISTICAL ANALYSIS

Data generated from the study were analyzed using SPSS 15 for windows. Parameters recorded on more than a single day (body weight, faecal egg counts, total white blood cell count, total red blood cell count, haemoglobin concentration) were analyzed by the repeated measures ANOVA in general linear model (GLIM) (Crawley, 1993) while those generated on a single day (Worm burden) were analyzed by one way ANOVA. Log probit analysis

(Finney, 1971) was used to determine the median lethal concentration (LC_{50}) of the extract and Albendazole in the egg hatch assay and *in vitro* Larva test. Variant means were separated by the Duncan's multiple range test and probabilities (p) of 0.05 or less were considered significant.

CHAPTER FOUR

RESULTS

4.1 PLANT EXTRACTION

The results of the plant extraction showed that the 300 grams of the dry plant material yielded 64.5 grams of extract. Therefore, the percentage yield of the plant extract was 21.5% (w/w).

4.2 PHYTOCHEMICAL ANALYSIS

The results of the phytochemical analysis of the methanol extract of *Duranta erecta* fruit is expressed in Table 1. The results showed that the methanol extract of *Duranta erecta* fruit contains several chemical components including tannins, flavonoids, glycosides, terpenes, polyuronides and saponins. However, the results showed that the plant extract did not contain any alkaloids, carbohydrates or starch.

4.3 ACUTE TOXICITY

Following the administration of graded doses (1000, 1600, 2900 and 5000 mg/kg bw respectively) of the methanol extract of *Duranta erecta* fruits to mice, no clinical signs of toxicity or mortality were observed in all treatment groups after 24 hours. Therefore, the LD₅₀ of the methanol extract of *Duranta erecta* fruit in mice appears to be greater than 5000 mg/kg and thus, according to the US EPA Classification, the extract is rated Category 4 as not acutely toxic i.e Unlikely to present acute hazard for normal use.

Table 1: Phytochemical Constituents of the Methanol Extract of *Duranta erecta* fruits

TESTS FOR :	INFERENCE
Alkaloids	-
Flavonoids	+
Carbohydrates	-
Starch	-
Tannins	+
Glycosides	+
Terpenes	+
Polyuronides	+
Saponins	+

4.4 IN VITRO ANTHELMINTHIC STUDIES

4.4.1 EGG HATCH ASSAY

Tables 2 and 3 show the effect of varied concentrations of the methanol extract of *Duranta erecta* fruits and Albendazole respectively on the embryonation of *Heligmosomoides bakeri* eggs. The percentage reduction in egg hatch by the control (de-ionized water) was 0%. A log₁₀ dose probit analysis showed that the methanol extract of *Duranta erecta* fruit had an LC₅₀ value of 0.796 mg/ml against *Heligmosomoides bakeri* eggs while Shanuzole® (25%w/w Albendazole) had an LC₅₀ value of 0.193µg/ml against the same eggs.

4.5 IN VIVO ANTHELMINTHIC STUDIES

4.5.1 CLINICAL SIGNS

The results obtained from the study showed that the 100 L₃ infective dose of *Heligmosomoides bakeri* larvae used in the study did not produce any overt clinical disease in the mice. There was slight weight loss and transient mucoid diarrhoea observed in some mice following infection. However this was not sustained throughout the experiment.

The effect of the methanol extract of *Duranta erecta* fruit on the body weight of mice infected with *Heligmosomoides bakeri* is illustrated in Figure 1. There were no significant ($p>0.05$) variations in the body weights of all the groups throughout the period of the study.

Table 2: Results of Egg Hatch Assay of *Heligmosomoides bakeri* Eggs Incubated with Graded Concentrations of *Duranta erecta* Methanol Fruit Extract

WELL NO	HATCHING (%)	PROBIT (HATCHING)	CONCENTRATION OF <i>DURANTA</i> <i>ERECTA</i> METHANOL FRUIT EXTRACT (mg/ml)
1	0	—	100.00
2	0	—	50.00
3	0	—	25.00
4	0	—	12.50
5	1	2.67	6.25
6	2	2.95	3.13
7	33	4.56	1.56
8	75	5.67	0.78
9	88	6.18	0.39
10	92	6.41	0.20
11	98	7.05	0.10
12	100	8.09	0.05
LC ₅₀ = 0.796mg/ml			

Table 3: Results of Egg Hatch Assay of *Heligmosomoides bakeri* Eggs Incubated with Graded Concentrations of Shanzole®

WELL NO	HATCHING (%)	PROBIT (HATCHING)	CONCENTRATION OF SHANUZOLE® (µg/ml)
1	21	4.19	25.00
2	24	4.29	12.50
3	32	4.53	6.25
4	46	4.90	3.13
5	51	5.03	1.56
6	64	5.36	0.78
7	77	5.74	0.39
8	86	6.08	0.20
9	88	6.18	0.10
10	94	6.55	0.05
11	96	6.75	0.02
12	99	7.33	0.01
LC ₅₀ = 0.193µg/ml			

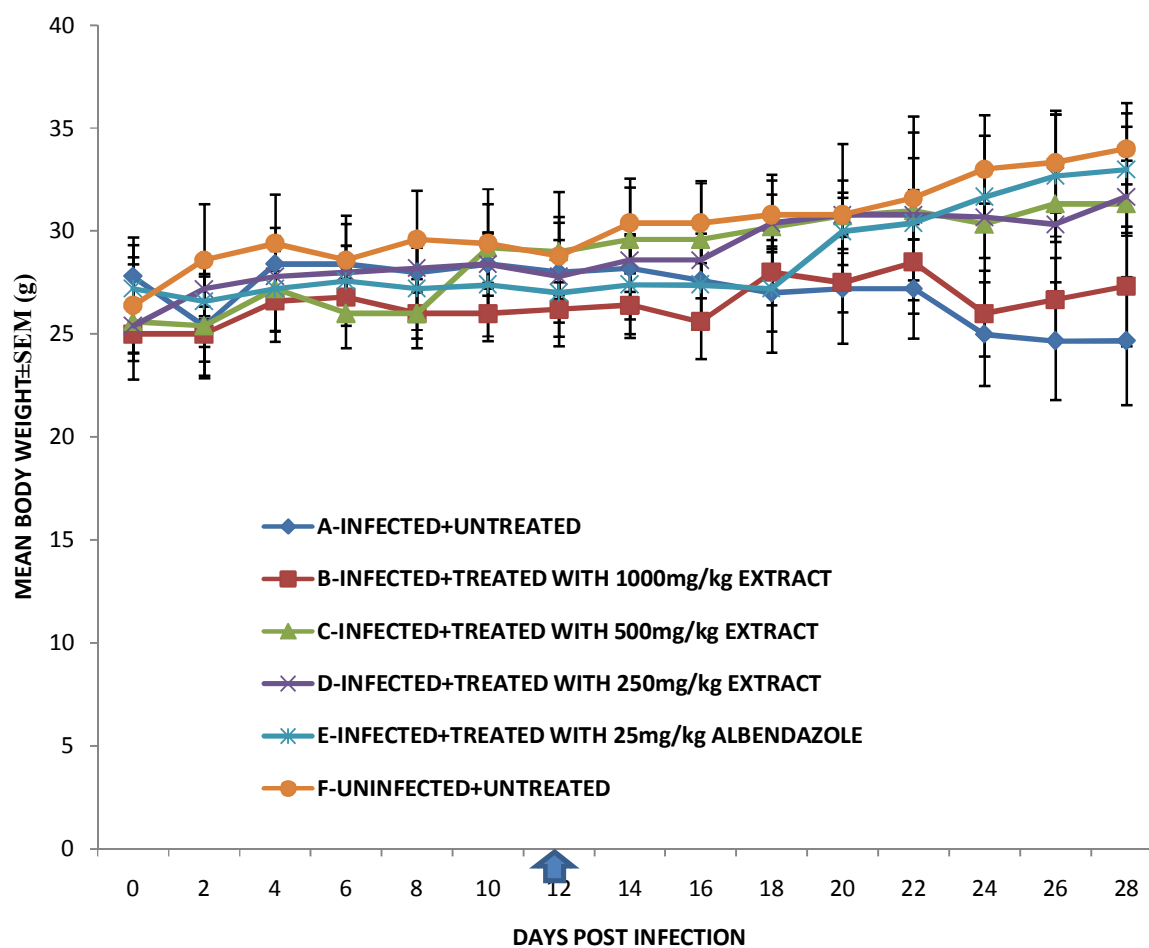


Figure 4.1: The Body Weight (g) of mice infected with *Heligmosomides bakeri* and treated with varied doses of *Duranta erecta* fruit extract.

(Day of treatment arrowed)

4.5.2 PARASITOLOGICAL EXAMINATION

4.5.2.1 FAECAL EGG COUNT

Figure 2 shows the effect of administration of the methanol extract of *Duranta erecta* fruits on faecal egg counts of mice infected with *Heligmosomoides bakeri*. Following infection of the mice in the various experimental groups, nematode eggs were first detected in the faeces from day 6 post-infection with a mean prepatent period of 7.08 ± 0.16 days and a range of 6 to 8 days for all infected groups. Thereafter, egg counts rose rapidly in all the infected groups. The rise continued steadily in the infected-untreated group without abatement to attain peak levels at the end of the experiment on day 28 post infection. A similar pattern was noted among the plant extract treated groups except that their egg counts remained generally higher than those of the untreated and Albendazole treated groups throughout the study period. On the other hand, treatment with 25 mg/kg bw Albendazole (Group E) suppressed the initial rise in faecal egg counts from day 14 post infection to the end of the experiment. On day 16 post infection, the mean egg counts observed in Group D (250mg/kg bw extract) was significantly ($p < 0.05$) higher than those of all other infected groups while the counts for Group E remained significantly ($p < 0.05$) lower than those of all the other treatment groups from day 18 post infection to the end of the study. It is noteworthy that treatment with either the extract or Albendazole did not completely clear faecal egg excretion in any of the treated groups throughout the study.

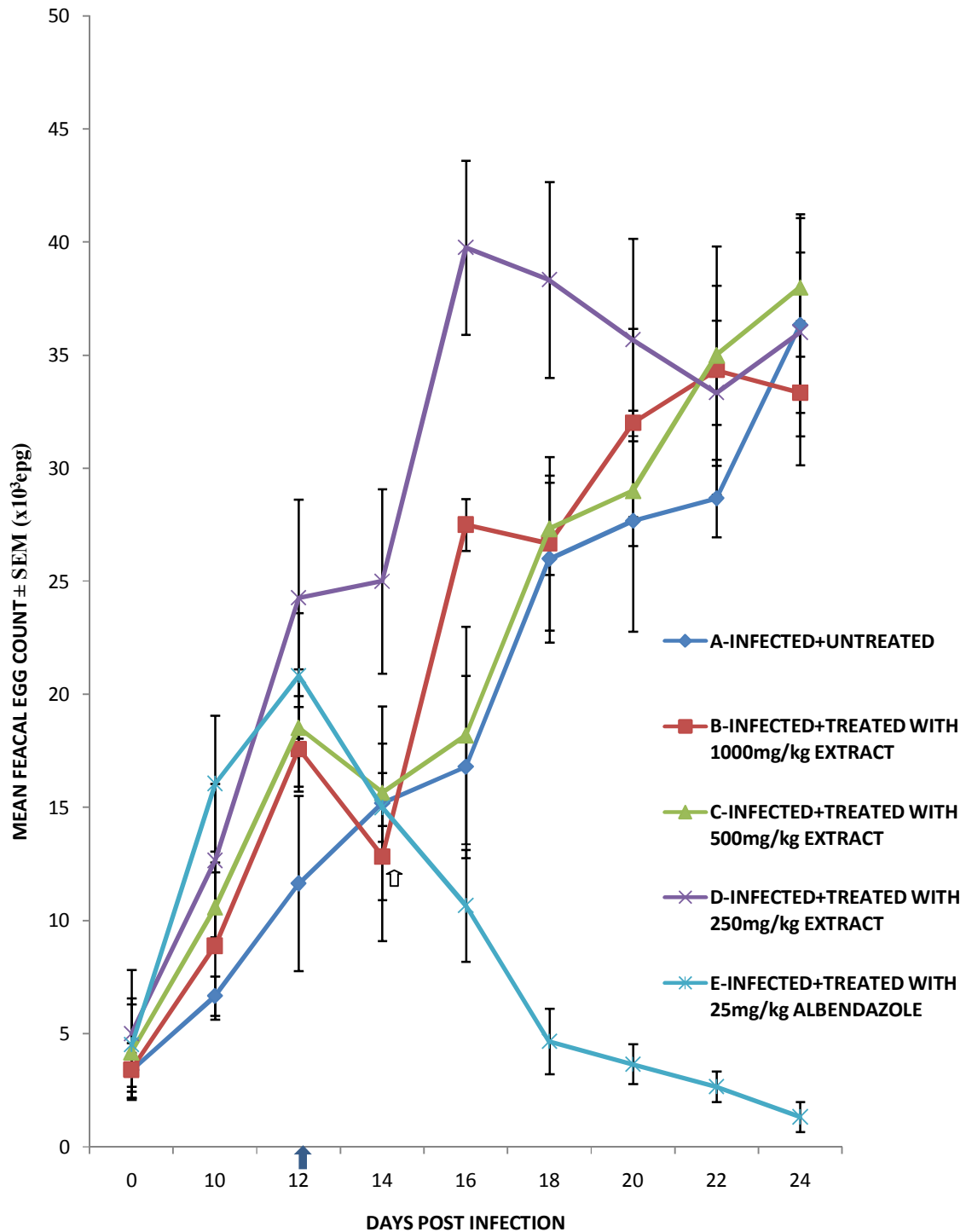


Figure 2: The Faecal egg count (x 10³ epg) of mice infected with *Heligmosomoides bakeri* and treated with varied doses of *Duranta erecta* methanol fruit extract.*

(Day of treatment arrowed)

4.5.2.2 ADULT WORM COUNT

Out of the 100 L₃ of *H. bakeri* used for the infection of each experimental group, the mean number of adult worms recovered at the end of the study on day 28 post infection were 55.2±0.51, 34.5±1.83, 48.6±2.10, 51.3±0.26 and 4.80±0.24 respectively for groups A, B, C, D and E. Consequently, the percentage worm establishment for the infected untreated group was 55%. The percentage worm recovery for various groups with regard to the infected untreated control group were 63.6%, 87.3%, 92.3% and 8.7% respectively for groups B, C, D and E.

The post mortem worm counts of the various groups are shown in Figure 3. Adult worm populations were relatively higher among the untreated group compared to the extract treated or Albendazole treated groups. Among the extract treated groups, a slight dose dependent anthelmintic effect was noted in worm numbers although with no significant ($p>0.05$) difference between the groups. On the other hand, adult worm numbers were significantly ($p<0.05$) lower in the albendazole treated than the extract treated groups.

There were also variations in the sexes of the worms recovered at the end of the study. Male worms were higher in number than female worms in the untreated group but this difference was not statistically significant ($p>0.05$). The same trend occurred in the extract treated group B (1000 mg/kg bw). However, the difference in the sexes was observed to be statistically significant ($p<0.05$). The reverse was observed in the extract treated groups C (500 mg/kg bw) and D (250 mg/kg bw) where the ratio of female worms was significantly ($p<0.05$) higher than that of male worms recovered. In the Albendazole treated group, there were no differences in the sexes of the worms recovered at the end of the study.

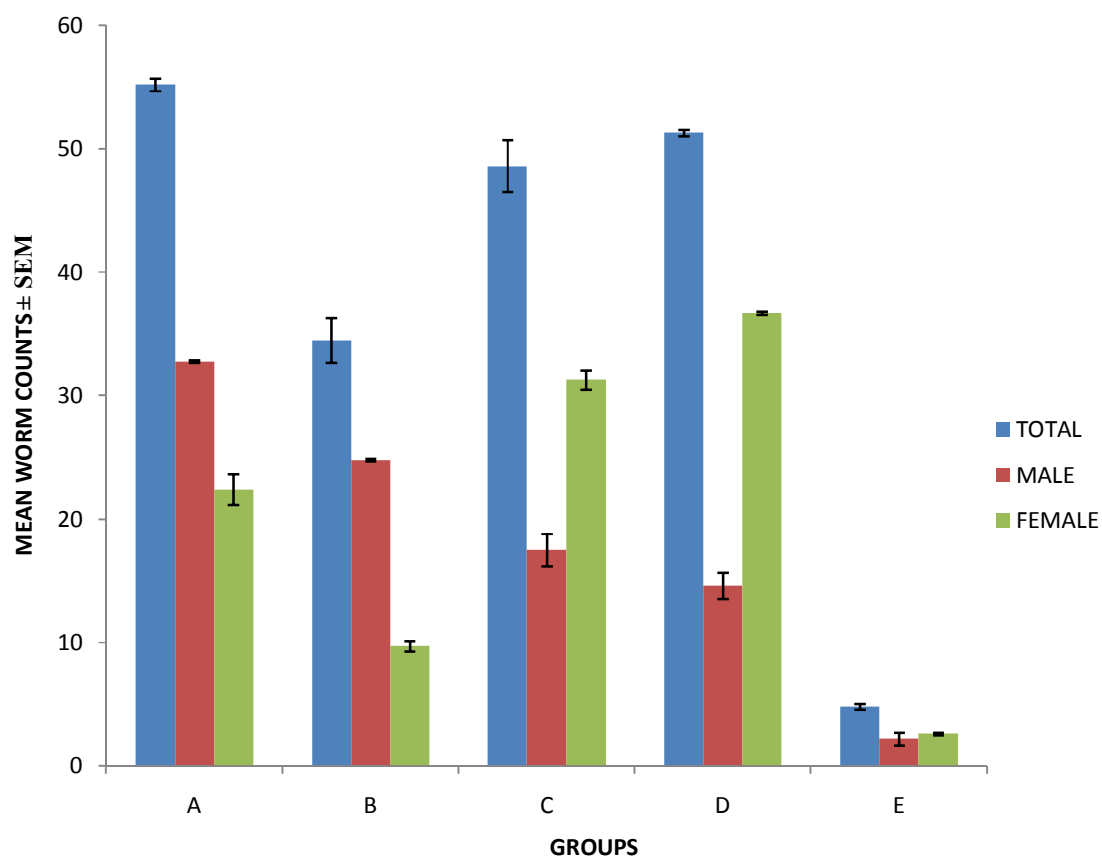


Figure 3: Mean post mortem Worm counts of mice infected with *Heligmosomoides bakeri* and treated with varied doses of *Duranta erecta* methanol fruit extract

Group A: Infected + Untreated.

Group B: Infected + treated with 1000 mg/kg bw Extract.

Group C: Infected + treated with 500 mg/kg bw Extract.

Group D: Infected + treated with 250 mg/kg bw Extract.

Group E: Infected + treated with 25 mg/kg bw Albendazole.

4.5.3 HAEMATOLOGY

4.5.3.1 PACKED CELL VOLUME (PCV)

The results of the mean packed cell volume of mice infected with *Heligmosomoides bakeri* and treated either with the Plant Extract or Albendazole are shown in Figure 4. Following infection of the mice, PCV values in all the infected groups began a general steady decline attaining their lowest levels on day 28 post infection as seen in the extract-treated and infected/untreated groups. Treatment with Albendazole reduced the rate and level of decline of PCV which recorded its lowest values by day 14 post infection, elevating the values to pre-infection values by the end of the experiment on day 28 post infection. There were no observed significant ($p>0.05$) variations in the PCV of the uninfected/untreated control group. On day 26 post infection, the mean PCV of the infected /untreated group was significantly ($p<0.05$) lower than those of the infected/treated and the uninfected/untreated groups. At the end of the study (day 28 post infection), groups A and B had significantly ($p<0.05$) lower PCV than all the other infected/treated groups.

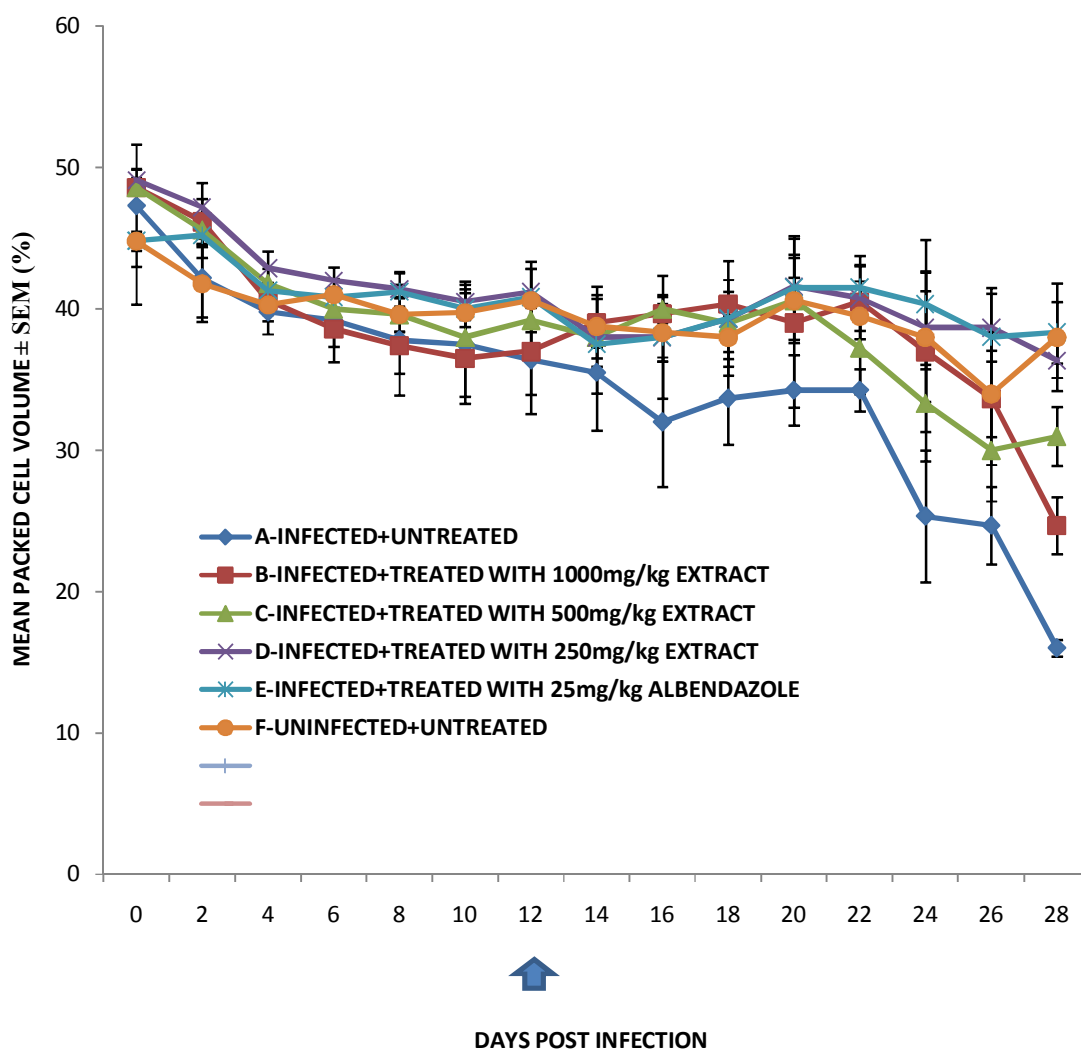


Figure 4: The packed cell volume (%) of mice infected with *Heligmosomoides bakeri* and treated with varied doses of *Duranta erecta* methanol fruit extract

(Day of treatment arrowed)

4.5.3.2 EFFECT ON HAEMOGLOBIN CONCENTRATION

Figure 5 shows the effect of treatment with the plant extract or Albendazole on the haemoglobin concentration of mice infected with *Heligmosomoides bakeri*. Following infection, there was a slight but steady decline in haemoglobin concentration in all infected groups when compared to the uninfected control group. Treatment with Albendazole (Group E) restored haemoglobin concentration to pre-infection values by day 28 post infection. Irrespective of treatment, between Day 0 and 12 post infection, there were no significant ($p < 0.05$) variations in the mean haemoglobin concentrations of all the infected mice groups. On day 16 post infection, the haemoglobin concentration of all infected groups were significantly ($p < 0.05$) lower than that of the uninfected control group. There was a progressive decrease ($p < 0.05$) in the mean haemoglobin concentration of the untreated control group (Group A) throughout the study. Among the groups treated with varied doses of the plant extract, there was also a gradual decrease in haemoglobin concentration throughout the study period but this decrease was not as marked as recorded in the untreated control group. By Day 28 post infection, the mean haemoglobin concentration of all the plant extract-treated groups were similar ($p > 0.05$) but significantly ($p < 0.05$) higher than that of the infected/untreated (Group A). The mean haemoglobin values of the albendazole treated group remained significantly ($p < 0.05$) higher than those of the extract-treated groups at the end of the study.

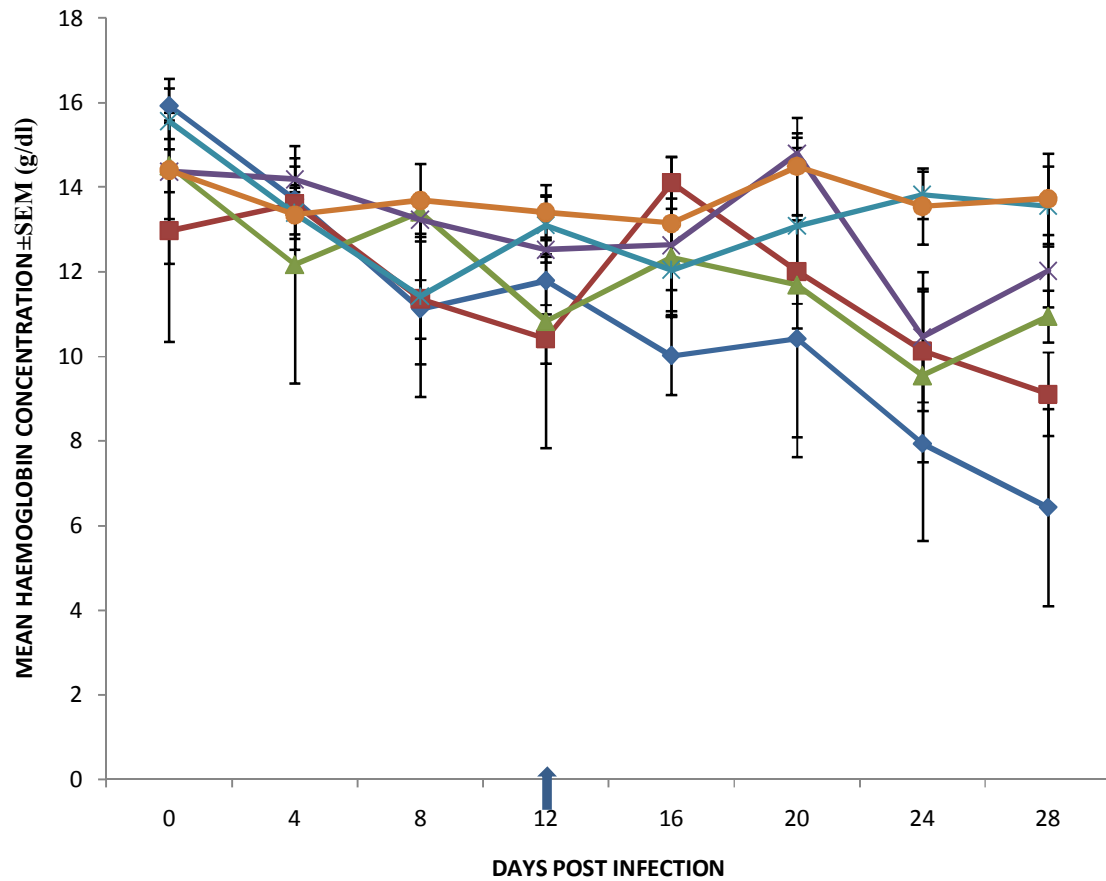


Figure 5: The Haemoglobin concentration (g/dl) of mice infected with *Heligmosomoides bakeri* and treated with *Duranta erecta* methanol fruit extract

Group A: Infected + Untreated.
Group B: Infected + treated with 1000 mg/kg bw Extract.
Group C: Infected + treated with 500 mg/kg bw Extract.
Group D: Infected + treated with 250 mg/kg bw Extract.
Group E: Infected + treated with 25 mg/kg bw Albendazole.
 (Day of treatment arrowed)

4.5.3.3 EFFECT ON RED BLOOD CELL COUNTS

The mean red blood cell counts of the experimental animals are illustrated in Figure 6. The results showed that following infection, the red blood cell counts of the uninfected control group (F) fluctuated within their pre-infection levels throughout the study with no significant ($p>0.05$) variations while those of the infected groups decreased. Compared to the uninfected/untreated control, the red blood cell counts of the infected/untreated group (A) showed a progressive decline ($p<0.05$) throughout the period of the study. However, treatment with the extract reduced rate of decline of red blood cell counts in a dose dependent manner, the lower doses producing greater effect when compared with the infected/untreated group. Treatment with albendazole returned the red blood cell counts to their pre-infection values from day 16 post infection. At the end of the study (day 28 post infection), Groups A and B had significantly ($p<0.05$) lower red blood cell counts than all the other infected groups and the uninfected/untreated control group.

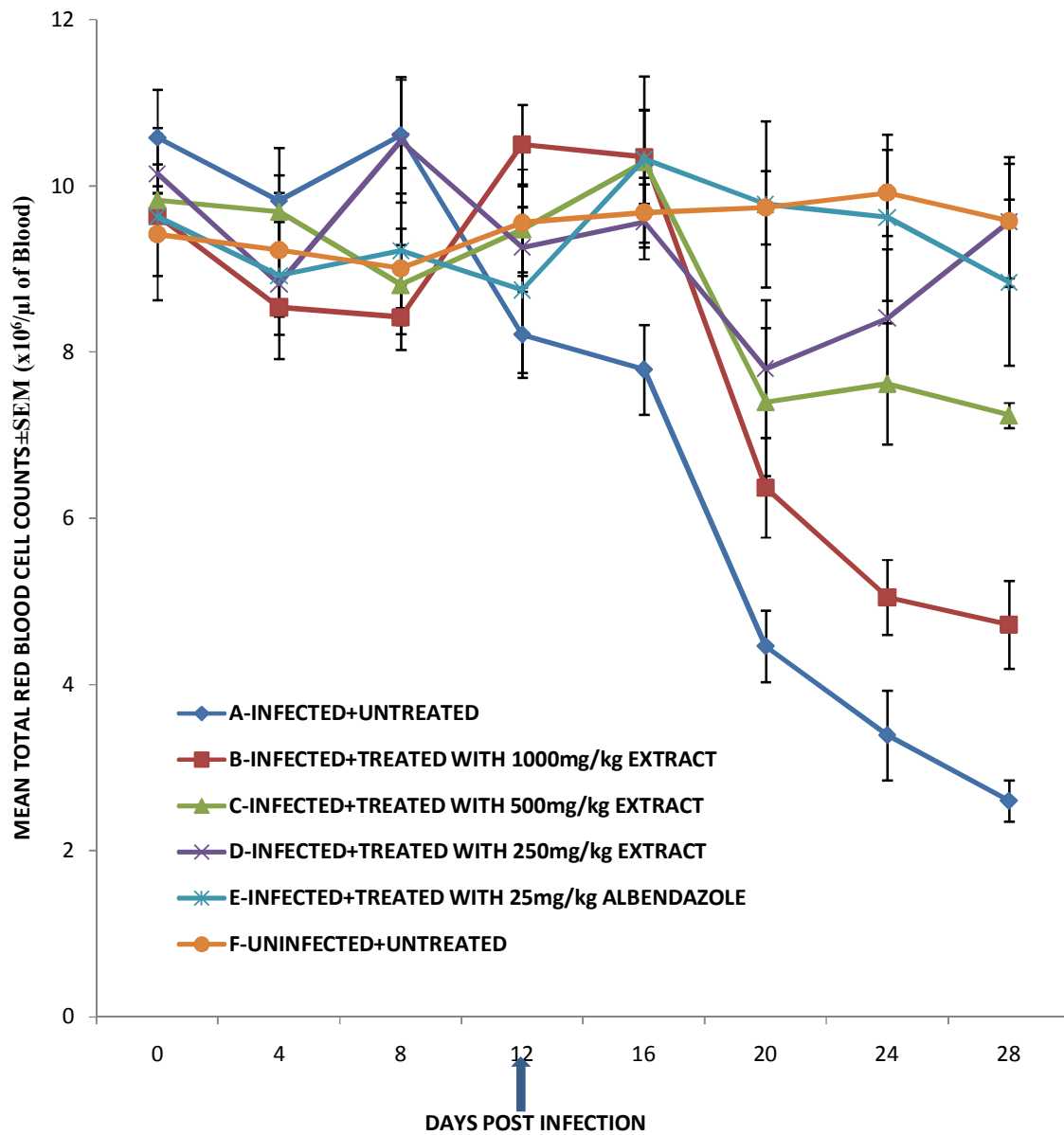


Figure 6: Red blood cell counts ($\times 10^6/\mu\text{l}$) of mice infected with *Heligmosomoides bakeri* and treated with varied doses of *Duranta erecta* methanol fruit extract or albendazole and their controls.

(Day of treatment arrowed)

4.5.3.4 EFFECT ON RBC INDICES

4.5.3.4.1 MEAN CORPUSCULAR VOLUME (MCV)

The MCV of the uninfected/untreated group remained within their normal pre-infection values without any significant ($p>0.05$) variations throughout the study period (Figure 7). On the other hand, following infection, the MCV of all the infected groups rose rapidly and remained above their pre-infection levels throughout the study except in the groups treated with 500 mg/kg extract and albendazole where the values returned to their pre-infection levels similar to the uninfected/untreated group by day 24 post infection. The MCV of the infected/untreated group remained generally lower than those of all the infected and treated groups (extract and albendazole) until day 20 when it became higher and rose to a peak on day 24 post infection. On the other hand, all the other infected groups had their highest MCV levels on day 12 post infection before returning to lower levels maintained for the rest of the study period. In general, the MCV of all the infected groups were significantly ($p<0.05$) higher than that of the uninfected/untreated group on day 12 post infection whereas the values for those treated with either albendazole or the extract (1000 mg/kg) were also significantly ($p<0.05$) higher than those of the rest of the infected groups and the uninfected/untreated groups by day 24 post infection.

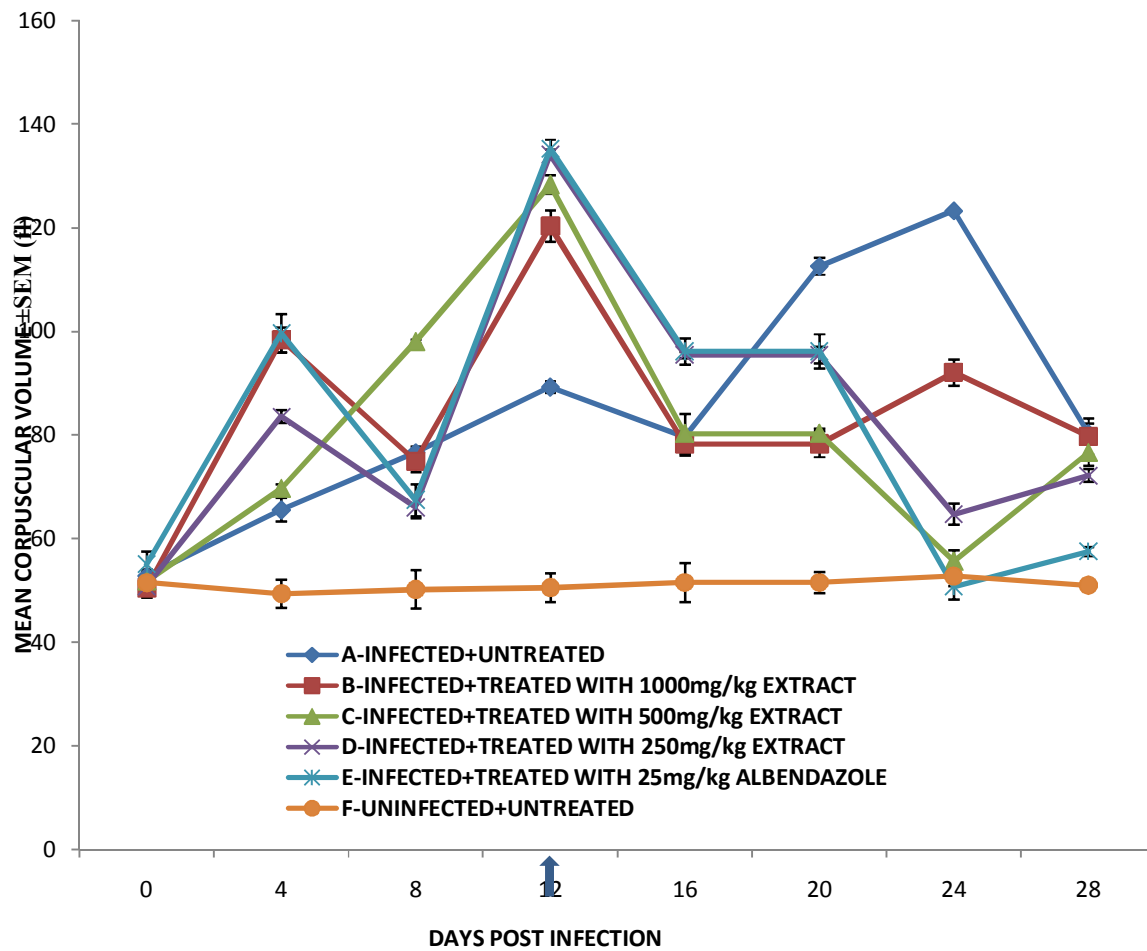


Figure 7: Mean Corpuscular Volume (fl) of mice infected with *Heligmosomoides bakeri* and treated with varied doses of *Duranta erecta* methanol fruit extract

(Day of treatment arrowed)

4.5.3.4.2 MEAN CORPUSCULAR HAEMOGLOBIN (MCH)

The MCH of the uninfected/untreated group remained within their normal pre-infection values without any significant ($p>0.05$) variations throughout the study period (Figure 8). On the other hand, the MCH of the infected/untreated group rose rapidly after infection and remained significantly ($p<0.05$) higher than that of the uninfected/untreated control group from day 16 to the end of the study. Compared to the uninfected/untreated group, MCH values also rose rapidly in all the treated groups (extract or albendazole) following infection to attain peak levels by day 12 ($p<0.05$) before returning to lower levels maintained for the rest of the study period. By day 24 post infection, the mean corpuscular haemoglobin values of the untreated control group was significantly ($p<0.05$) higher than all groups except group B which received 1000mg/kg bw of the extract. Group B was however not different from the other groups.

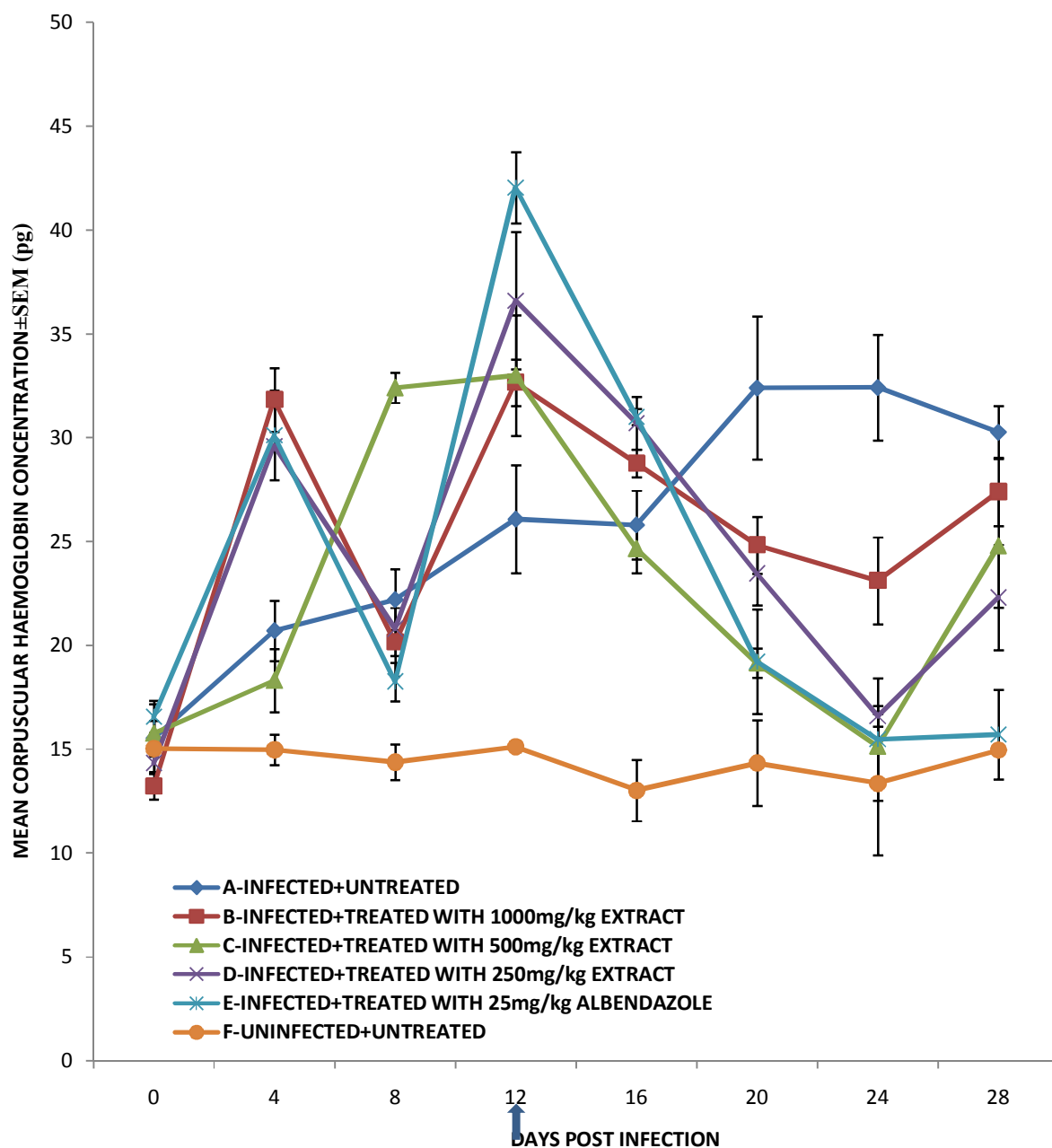


Figure 8: Mean haemoglobin concentration (pg) of mice infected with *Heligmosomoides bakeri* and treated with varied doses of *Duranta erecta* methanol fruit extract

(Day of treatment arrowed)

4.5.3.4.3 MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC)

The results of the mean corpuscular haemoglobin concentration are shown in Figure 9. The MCHC of all the experimental mice varied throughout the experiment but without any significant ($p>0.05$) changes between all groups including the uninfected/untreated control.

4.5.3.5 TOTAL WHITE BLOOD CELL COUNT

The mean total WBC counts of the uninfected/untreated group remained within their normal pre-infection values without any significant ($p>0.05$) variations throughout the study period (Figure 10). On the other hand, following infection, the WBC count of the infected/untreated group rose rapidly to higher levels maintained to the end of the study. However, treatment with the extract or albendazole resulted in a rapid decline in the WBC values of such groups and by day 24 post infection, the WBC counts had returned to their normal pre-infection levels in the groups treated with albendazole or the extract at 250 and 500 mg/kg body weight. By day 24 post infection, the total white blood cell count of Group A was significantly ($p<0.05$) higher than those of groups C, D, E and F but was not significantly ($p>0.05$) different from that of group B.

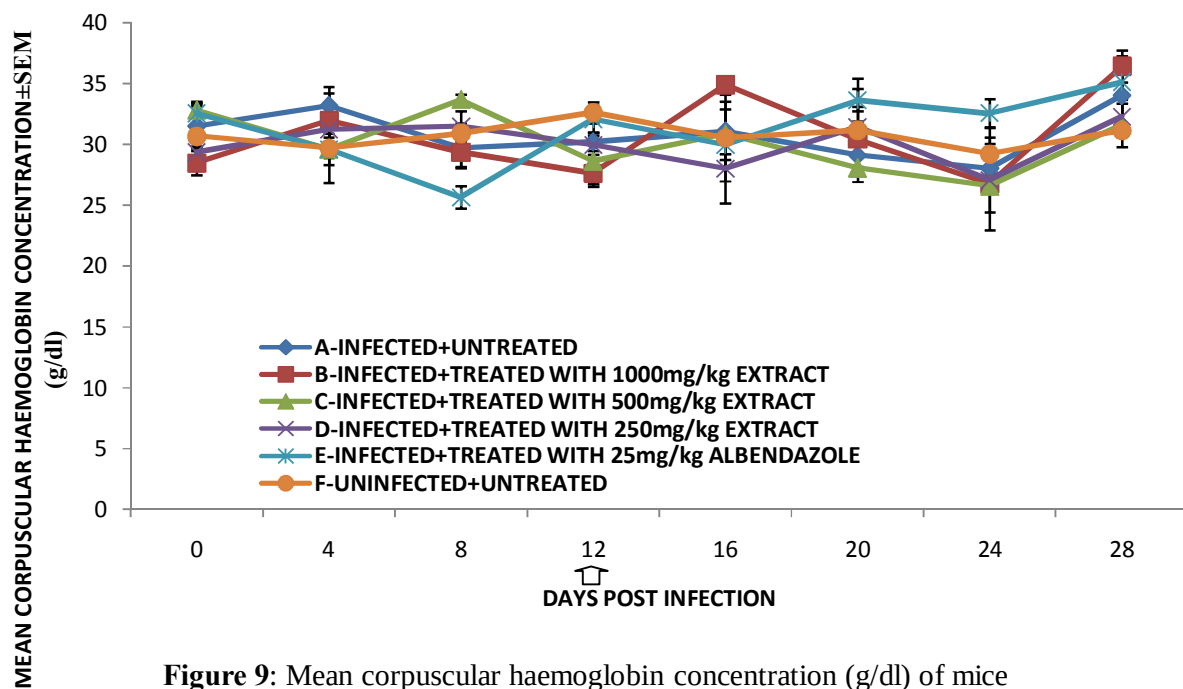


Figure 9: Mean corpuscular haemoglobin concentration (g/dl) of mice infected with *Heligmosomoides bakeri* and treated with varied doses of *Duranta erecta* methanol fruit extract

(Day of treatment arrowed)

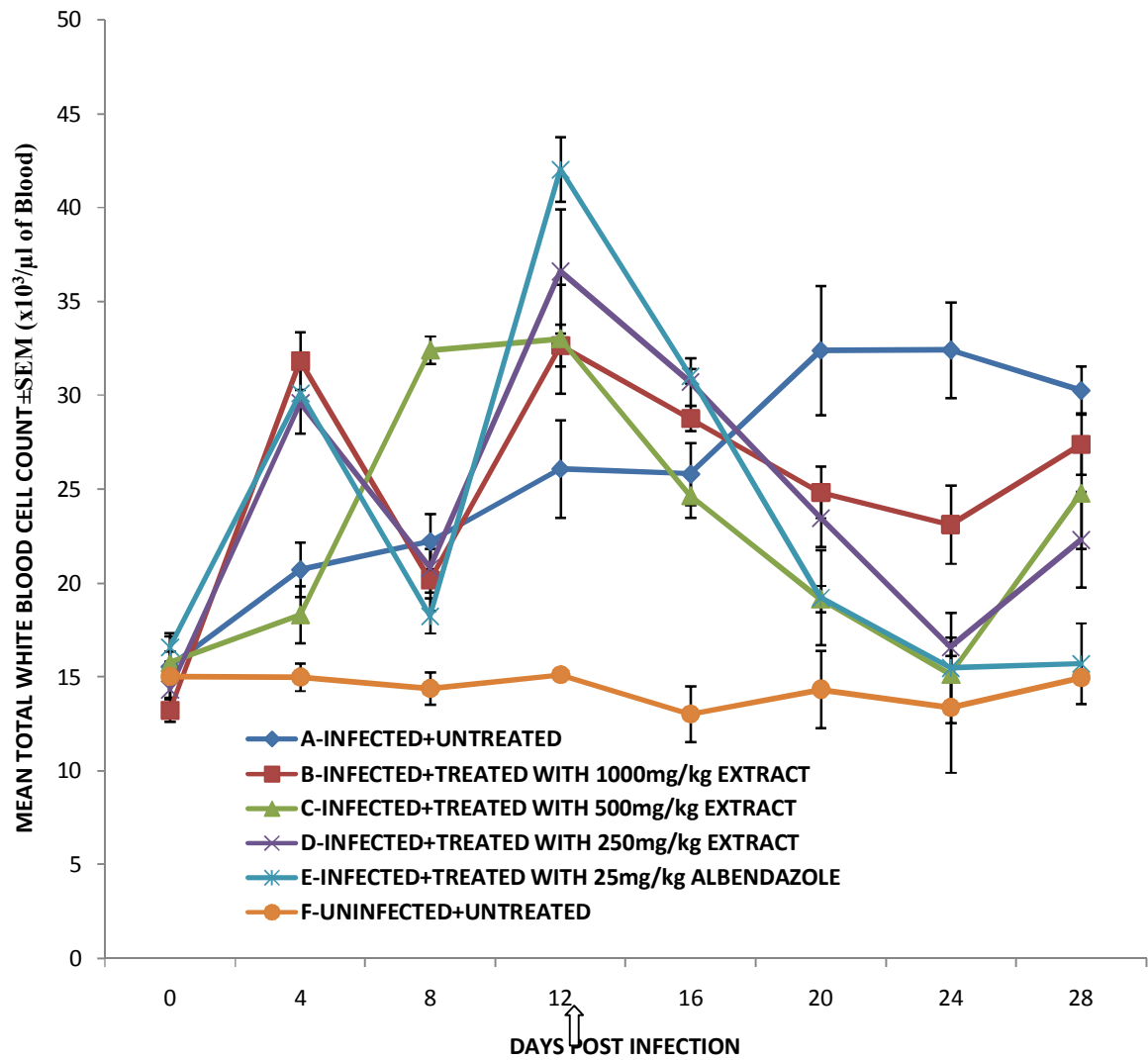


Figure 10: The mean total white blood cell counts ($\times 10^3/\mu\text{l}$) of mice infected with *Heligmosomoides bakeri* and treated with varied doses of *Duranta erecta* methanol fruit extract

(Day of treatment arrowed)

CHAPTER FIVE

DISCUSSION

5.1 DISCUSSION

The results of the phytochemical analysis revealed that the methanol extract of *Duranta erecta* fruit contains several active chemical components including flavonoids, tannins, glycosides, terpenes, polyuronides and saponins. Some of these components obtained during the study have previously been reported to be present in other plants (Okafor and Ejiofor, 1996; Onyeyili *et al.*, 2001; Farombi, 2003; Eleyinmi, 2007).

The results of the acute toxicity of the plant extract revealed that the methanol extract of *Duranta erecta* fruit did not cause mortalities in treated mice and was not toxic to mice even at high doses of 5000 mg/kg bw. According to the US EPA Classification of toxic substances, orally administered substances with LD₅₀ values above 5000 mg/kg bw are considered safe for oral use. Consequently, the extract appears to be safe at the doses used in the mice during this study.

In vitro anthelmintic studies of plants provide preliminary investigations into the anthelmintic properties of such plants. The main advantage of using *in vitro* assays to test the anthelmintic properties of plant extracts is that the study is done at cheaper cost than *in vivo* tests, and there is rapid turn-over which allows large scale screening of plants (Enejoh *et al.*, 2015). As a matter of fact, most anthelmintic screening of plants reported so far have been based on *in vitro* studies (Mali *et al.*, 2007). The results of the *in vitro* evaluation of the anthelmintic effect of the methanol extract of *Duranta erecta* fruit revealed that the extract had suppressive effects on embryonation of *Heligmosomoides bakeri* eggs at 0.796 mg/ml.

The anthelmintic effect recorded by the plant extract may have been due to the synergistic action of the different active components of the plant extract, penetrating the helminth egg shell, and interfering with segmentation of blastomeres thereby inhibiting egg development and hatch. Previous studies on other plants have shown that plant extracts usually produce concentration-dependent effects when tested on helminth eggs (Ademola and Eloff, 2011; Kollins *et al.*, 2012). Such trials of medicinal plants have shown that the presence of secondary metabolites like alkaloids, saponins, polyphenols, carotenoids, triterpenes, saponosides, embeline, sesquiterpenolactones may be responsible for their anthelmintic effects (Suleiman *et al.*, 2005; Cock, 2011; Kollins *et al.*, 2012).

For instance, tannins have been reported to have anthelmintic properties (Ngongeh and Fakae, 2011) possibly due to their ability to bind free proteins in the gastrointestinal tract of the host animal or glycoproteins in the cuticle of parasites and cause mortality (Athanasiadou *et al.*, 2007). Tannins were detected in the extract of *D. erecta* and may have been responsible for its anthelmintic activity. Saponins, also present in the extract have also been reported to have anthelmintic activity. They are known to act by destabilizing the membranes and increasing cell permeability of helminth parasites (Price *et al.*, 1987). Some other members of the family *Verbenacea* have also been reported to have anthelmintic properties (Al-Amin *et al.*, 2007; Paria *et al.*, 2012; Islam *et al.*, 2013).

The anthelmintic activity recorded for the methanol extract of *D. erecta* fruit in this study was however very weak when compared with Albendazole which gave an LC₅₀ of 0.193µg/ml. Albendazole showed significantly higher anthelmintic activity than the methanol extract of *Duranta erecta* fruit in this study even though the concentration of Albendazole used was much lower. This may have been as a result of the absence of some secondary metabolites like alkaloids. Alkaloids are nitrogenous compounds which occur in plants and have been reported to be responsible for a plant's anthelmintic activity (Okwu,

2001). In synergy with tannins, and glycosides and other metabolites, alkaloids have been reported to produce strong anthelmintic effect against various nematode parasite stages (Martin, 1989; Roy and Tandon, 1996). Consequently, the absence of alkaloids from the extract of *D. erecta* may be responsible for the very weak anthelmintic effect observed during this study. This is in contrast to the findings of Nikkon *et al.* (2008) who reported a positive anthelmintic effect by the methanol extract of *Duranta erecta* leaves. The phytochemical screening of the leaves showed the presence of alkaloids, tannins, saponins, flavonoids, and glycosides and these were suggested to have been responsible for the strong anthelmintic effect recorded by the leaf extract (Ogbuagu *et al.*, 2015).

Inhibition on egg production is an important aspect of the activity of some antinematodals such as benzimidazoles and phenothiazines. Albendazole is a broad spectrum anthelmintic reputed for its efficacy against all parasite stages (Riviere and Papich, 2009). They have broad spectrum of activity on many parasites of medical and veterinary importance (Geary *et al.*, 1999). Previous studies showed a 94.1% reduction in faecal egg output of sheep treated with this proprietary anthelmintic against mixed nematode species (*Haemonchus contortus*, *Trichostrongylus colubriformis*, *Trichostrongylus axei*, *Oesophagostomum columbianum*, *Strongyloides papillosus* and *Trichuris ovis*) at the dose 5 mg kg bwt (Onyeyili *et al.*, 2001; Githiori *et al.*, 2004).

The results obtained from the study showed that the infective dose of *Heligmosomoides bakeri* larvae used in the study did not produce overt clinical disease in mice as there were no obvious clinical signs besides the slight weight loss and transient mucoid diarrhoea observed in some of the infected mice. These findings correspond with the reports of Fakae (1993) and Monroy and Enriquez (1992).

The results of the study showed that the methanol extract of *Duranta erecta* fruits showed very weak anthelmintic effect *in vitro*. Also, there was no significant anthelmintic activity recorded *in vivo* in the treated animals as the results showed that the plant extract did not cause significant reductions in egg counts in any of the treated groups as was recorded with the Albendazole treated group. The slight depression in faecal egg output observed in the mice groups treated with 500 and 1000 mg/kg bw of the plant extract by day 2 post treatment was not sustained as egg counts continued to rise in all treated groups to values comparable to the untreated control group by the end of the experiment. This observation further attested to the poor anthelmintic property of the plant extract *in vitro*.

Regardless of the dose used in the study, worm burdens in the groups treated with the plant extract were comparable to the untreated control group thus, suggesting that the plant extract was unable to eliminate adult worms from the gastrointestinal tract of the host animal. This is in contrast to Albendazole which produced 92% reduction in worm burden in infected animals further alluding to the ineffective nematicidal properties of the plant extract *in vivo*. The weak anthelmintic effect observed *in vitro* in contrast to the *in vivo* result may have been due to direct exposure of the parasite stages to the chemical compounds in the plant extract as against what obtains *in vivo*, where the host's physiological processes contribute to the outcome of a compound introduced into the body system.

Blood is a tissue which consists of fluid plasma in which are suspended a number of formed elements (erythrocytes, leucocytes and thrombocytes). Its primary function is to provide a link between the various organs and cells of the body, and to maintain a constant cellular environment by circulating through every tissue, delivering nutrients to them and removing waste products (Bowman and Rand, 1980). The blood cells exist at fairly constant levels, suggesting the existence of feedback mechanisms for the cells (Guyton and Hall,

2006). Consequently, variations in blood parameters are used as viable indicators of different physiologic activities occurring in the body system (Egbe-Nwiyi *et al.*, 2012).

Anaemia is the major pathological effect of *Heligmosomoides bakeri* infection in mice and this is related to the blood loss in the intestine due to the feeding habits of adult parasites (Soulsby, 1982; Wabo *et al.*, 2010). Similarly, a decrease in erythrocytic values pre-treatment suggestive of anaemia was observed in all groups infected by the parasite when compared with the uninfected control group. Despite the decrease in erythrocytic values, treatment with the extract was however observed to slow the depression in erythrocytic values including packed cell volume (PCV), haemoglobin concentration (Hb) and red blood cell (RBC) counts compared with the infected/untreated group. This may be suggestive of a possible stimulatory effect on the bone marrow by the plant extract, leading to erythropoiesis to compensate for the loss of blood cells caused by the haematophagous activity of the parasite in their hosts. This is further validated by the presence of large red blood cells as reflected from the MCV values during the course of the study. This is also suggestive of a macrocytic hypochromic anaemia as inferred from the results of the red blood cell indices.

Following infection with the parasite, there was marked leucocytosis in all infected groups when compared with the uninfected control group. The observed leucocytosis was consistent with reported universal features of the host response to helminth infections (Fakae *et al.*, 1999; Zajac and Garya, 2006). Following administration of the varied doses of the plant extract, there was a reversal of the observed leucocytosis to near pre-infection values especially in the groups treated with 250 and 500 mg/kg bw of the extract.

There was a gradual drop in body weight of mice in all infected groups in comparison to the uninfected control group post infection. This loss in weight could be attributed to infection with the parasite. Weight loss is described as one of the common clinical signs of

helminth infections and known to occur due to anorexia or inappetence, malnourishment or blood or protein loss associated with nematode infections (Hotez *et al.*, 2004; Behnke *et al.*, 2009; Ngongeh and Fakae, 2011). Most of the mice in this study recorded a gradual weight gain following treatment with the plant extract or Albendazole. This suggests that the plant extract and Albendazole were able to positively reverse the effect of the infection on body weight. It also suggests that the plant extract had some positive effect on weight gain by infected animals.

5.2 CONCLUSION/RECOMMENDATION

The results of this study suggest that the methanol extract of *Duranta erecta* fruit has poor anthelmintic properties against the murine nematode *Heligmosomoides bakeri* as it was unable to eliminate the faecal egg output or adult worms in the gastrointestinal tract of the animals at the doses used in the study. The results however showed possible positive effects on weight gain in infected animals. It is therefore recommended that the plant extract be further explored for other useful effects than as an anthelmintic.

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APPENDIX

Table 1. The body weight (g) of mice infected with *Heligmosomoides bakeri* and treated with varied doses of *Duranta erecta* fruit extract [treatment was done on day 12].

Exptl. period (days)	Mean body weight (g), with standard error in brackets					
	Group A (untreated control)	Group B (1000mg/kg extract)	Group C (500mg/kg extract)	Group D (250mg/kg extract)	Group E (Albendazole)	Group F (uninfected control)
0	27.80 (1.53)	25.00 (1.30)	25.60 (2.80)	25.40 (1.29)	27.20 (2.50)	26.40 (2.34)
2	25.40 (2.54)	25.00 (1.34)	25.40 (2.42)	27.20 (1.46)	26.60 (2.20)	28.60 (2.73)
4	28.40 (1.78)	26.60 (1.47)	27.20 (2.56)	27.80 (1.24)	27.20 (2.03)	29.40 (2.40)
6	28.40 (1.97)	26.80 (1.39)	26.00 (1.67)	28.00 (1.30)	27.60 (1.69)	28.60 (2.16)
8	28.00 (1.82)	26.00 (1.22)	26.00 (1.67)	28.20 (1.16)	27.20 (1.99)	29.60 (2.38)
10	28.40 (1.54)	26.00 (1.34)	29.20 (2.85)	28.40 (1.17)	27.40 (2.50)	29.40 (1.91)
12	28.00 (2.43)	26.20 (1.32)	29.00 (2.92)	27.80 (1.07)	27.00 (2.59)	28.80 (1.91)
14	28.20 (2.48)	26.40 (1.57)	29.60 (2.54)	28.60 (1.25)	27.40 (2.38)	30.40 (2.16)
16	27.60 (2.29)	25.60 (1.81)	29.80 (2.85)	27.80 (1.07)	26.40 (1.99)	29.80 (1.93)
18	27.00 (2.88)	28.00 (1.58)	30.20 (2.54)	30.40 (1.40)	27.20 (2.08)	30.80 (1.66)
20	27.20 (2.65)	27.50 (1.44)	30.75 (3.50)	30.80 (1.07)	30.00 (1.63)	30.80 (1.66)
22	27.20 (2.40)	28.50 (1.85)	31.00 (2.55)	30.80 (1.20)	30.40 (4.39)	31.60 (3.98)
24	25.00 (2.52)	26.00 (2.08)	30.33 (4.33)	30.68 (0.67)	31.67 (2.96)	33.00 (2.65)
26	24.67 (2.85)	26.67 (2.03)	31.33 (4.33)	30.33 (0.58)	32.67 (3.18)	33.33 (2.33)
28	24.67 (3.10)	27.33 (2.91)	31.33 (3.76)	31.67 (1.76)	33.00 (3.22)	34.00 (1.73)

Table 2. The faecal egg count ($\times 10^3$ epg) of mice infected with *Heligmosomoides bakeri* and treated with varied doses of *Duranta erecta* fruit extract [treatment was done on day 12].

Exptl. period (days)	Means of faecal egg count ($\times 10^3$ epg) with standard error in brackets				
	Group A (untreated control)	Group B (1000mg/kg extract)	Group C (500mg/kg extract)	Group D (250mg/kg extract)	Group E (Albendazole)
0	3.39 (1.22)	3.42 (0.75)	4.20 (2.11)	5.01 (2.82)	4.52 (2.06)
10	6.67 (0.87)	8.89 (3.26)	10.60 (1.99)	12.67 (3.39)	16.07 (3.00)
12	11.65 (3.87)	17.59 (1.87)	18.53 (2.59)	24.27 (4.34)	20.83 (2.77)
14	15.20 (4.28)	12.83 (3.71)	15.67 (2.18)	25.00 (4.08)	15.00 (0.81)
16	16.80 (4.03)	27.50 (1.14)	18.20 (4.81)	39.75 (3.84)	10.67 (2.47)
18	26.00 (3.69)	26.67 (3.84)	27.33 (2.03)	38.33 (4.33)	4.67 (1.45)
20	27.67 (4.88)	32.00 (4.16)	29.00 (2.43)	35.67 (4.48)	3.67 (0.88)
22	28.67 (1.72)	34.33 (5.49)	35.00 (3.07)	33.33 (3.21)	2.67 (0.67)
24	36.33 (4.91)	33.33 (3.18)	38.00 (3.06)	36.00 (3.54)	1.33 (0.67)

Table 3. The Haemoglobin Concentration (g/dl) of mice infected with *Heligmosomoides bakeri* and treated with *Duranta erecta* fruit extract [Treatment was done on Day 12]

Exptl. period (days)	Mean Haemoglobin Concentration (g/dl) with standard error in brackets					
	Group A (untreated control)	Group B (1000mg/kg g extract)	Group C (500mg/kg extract)	Group D (250mg/kg extract)	Group E (Albendazole)	Group F (uninfected control)
0	15.93 (0.41)	12.97 (2.62)	14.51 (1.26)	14.38 (2.19)	15.56 (0.42)	14.40 (0.51)
4	13.71 (0.35)	13.61 (1.08)	12.17 (2.81)	14.19 (0.30)	13.39 (0.60)	13.35 (0.46)
8	11.12 (0.69)	11.36 (1.54)	13.41 (0.15)	13.24 (0.52)	11.42 (2.37)	13.69 (0.86)
12	11.79 (0.57)	10.42 (0.58)	10.83 (2.99)	12.52 (0.29)	13.10 (0.68)	13.41 (0.65)
16	10.02 (0.92)	14.11 (0.61)	12.35 (1.39)	12.63 (1.55)	12.04 (1.06)	13.15 (1.57)
20	10.42 (2.80)	12.01 (1.34)	11.69 (3.59)	14.80 (0.38)	13.09 (1.84)	14.49 (1.15)
24	7.94 (2.30)	10.13 (1.42)	9.55 (2.05)	10.46 (1.54)	13.82 (0.56)	13.55 (0.90)
28	6.43 (2.33)	9.11 (0.99)	10.95 (0.61)	12.02 (0.85)	13.55 (0.94)	13.73 (1.07)

Table 4. The Mean Corpuscular Haemoglobin (pg) of mice infected with *Heligmosomoides bakeri* and treated with the Plant Extract or Albendazole. Treatment was carried out on Day 12

Exptl. period (days)	Mean Corpuscular Haemoglobin (pg) with standard error in brackets					
	Group A (untreated control)	Group B (1000mg/kg extract)	Group C (500mg/kg extract)	Group D (250mg/kg extract)	Group E (Albendazole)	Group F (uninfected control)
0	15.54 (1.63)	13.22 (0.62)	15.76 (0.61)	14.34 (0.50)	16.58 (0.77)	15.03 (0.37)
4	20.71 (1.45)	31.82 (1.54)	18.31 (1.52)	29.60 (0.07)	30.11 (2.16)	14.98 (0.73)
8	22.21 (1.46)	20.16 (1.65)	32.41 (0.73)	20.80 (1.29)	18.25 (0.93)	14.38 (0.85)
12	26.08 (2.59)	32.66 (1.12)	33.00 (2.90)	36.60 (3.31)	42.03 (1.71)	15.12 (0.23)
16	25.80 (1.65)	28.77 (0.67)	24.66 (1.19)	30.70 (1.27)	31.01 (0.39)	13.02 (1.47)
20	32.39 (3.44)	24.83 (1.37)	19.15 (0.70)	23.46 (1.53)	19.23 (2.52)	14.34 (2.06)
24	32.41 (2.55)	23.12 (2.09)	15.14 (1.56)	16.60 (0.49)	15.48 (2.94)	13.36 (3.46)
28	30.26 (1.28)	27.40 (1.64)	24.79 (2.97)	22.31 (2.54)	15.71 (2.05)	14.97 (0.13)

Table 5. The Mean Corpuscular Haemoglobin Concentration (g/dl) of mice infected with *Heligmosomoides bakeri* and treated with the Plant Extract or Albendazole. Treatment was carried out on Day 12

Exptl. period (days)	Mean Corpuscular Haemoglobin Concentration (g/dl) with standard error in brackets					
	Group A (untreated control)	Group B (1000mg/k g extract)	Group C (500mg/kg extract)	Group D (250mg/k g extract)	Group E (Albendazole)	Group F (uninfected control)
0	31.49 (1.84)	28.45 (1.00)	32.82 (0.56)	29.36 (0.45)	32.52 (0.97)	30.66 (2.49)
4	33.18 (1.54)	31.98 (2.20)	29.60 (1.29)	31.23 (1.30)	29.60 (2.77)	29.70 (0.84)
8	29.71 (1.56)	29.36 (1.32)	33.68 (0.39)	31.48 (1.22)	25.65 (0.91)	30.90 (0.84)
12	30.19 (0.72)	27.61 (1.12)	28.60 (1.87)	29.93 (0.80)	32.07 (1.10)	32.60 (0.86)
16	31.05 (1.85)	34.90 (0.27)	30.86 (2.66)	28.01 (2.86)	29.94 (1.21)	30.52 (3.56)
20	29.09 (2.18)	30.42 (1.45)	28.04 (0.09)	31.43 (3.95)	33.64 (0.90)	31.15 (1.92)
24	28.01 (2.03)	26.74 (3.82)	26.57 (2.18)	27.08 (0.52)	32.54 (1.20)	29.21 (2.18)
28	34.01 (3.23)	36.40 (1.33)	31.58 (1.79)	32.25 (1.40)	35.10 (0.85)	31.12 (0.35)

Table 6. The Mean Corpuscular Volume (fl) of mice infected with *Heligmosomoides bakeri* and treated with the Plant Extract or Albendazole. Treatment was carried out on Day 12

Exptl. period (days)	Mean Corpuscular Volume (fl) with standard error in brackets					
	Group A (untreated control)	Group B (1000mg/k g extract)	Group C (500mg/kg extract)	Group D (250mg/k g extract)	Group E (Albendazole)	Group F (uninfected control)
0	52.80 (0.72)	50.49 (1.45)	51.83 (2.21)	50.84 (2.25)	55.04 (2.48)	51.49 (0.34)
4	65.57 (2.28)	98.35 (2.38)	69.67 (0.82)	83.60 (1.24)	99.62 (3.70)	49.35 (2.74)
8	76.63 (1.13)	74.98 (2.18)	98.04 (0.37)	66.10 (2.14)	67.45 (3.09)	50.20 (3.69)
12	89.29 (1.13)	120.31 (3.02)	128.33 (1.80)	134.15 (0.93)	135.25 (1.78)	50.55 (2.74)
16	79.66 (1.27)	78.27 (2.24)	80.29 (3.82)	95.47 (0.59)	96.14 (2.49)	51.55 (3.74)
20	112.61 (1.65)	78.50 (2.53)	67.37 (0.90)	68.82 (1.61)	59.60 (3.33)	50.96 (2.04)
24	123.29 (0.15)	92.10 (2.54)	55.62 (2.09)	64.71 (2.02)	50.75 (2.47)	52.81 (1.99)
28	80.24 (2.03)	79.76 (3.41)	76.63 (2.60)	72.23 (1.27)	57.51 (0.92)	51.00 (1.09)

Table 7. The packed cell volume (%) of mice infected with *Heligmosomoides bakeri* and treated with varied doses of *Duranta erecta* fruit extract [treatment was done on day 12).

Exptl. period (days)	Mean PCV (%) with standard error in brackets					
	Group A (untreated control)	Group B (1000mg/kg g extract)	Group C (500mg/kg extract)	Group D (250mg/kg extract)	Group E (Albendazole)	Group F (uninfected control)
0	47.30 (4.32)	48.60 (1.33)	48.60 (0.51)	49.10 (0.75)	44.80 (0.66)	44.80 (4.48)
2	42.20 (2.77)	46.20 (1.59)	45.60 (1.21)	47.20 (1.74)	45.20 (1.59)	41.80 (2.71)
4	39.80 (1.59)	40.60 (1.03)	41.80 (1.07)	42.90 (1.19)	41.30 (0.54)	40.30 (1.18)
6	39.20 (1.88)	38.60 (2.34)	40.00 (0.84)	42.00 (0.95)	40.80 (0.73)	41.00 (1.14)
8	37.80 (3.92)	37.40 (1.97)	39.60 (1.17)	41.40 (1.21)	41.20 (1.32)	39.60 (1.25)
10	37.50 (4.21)	36.50 (2.72)	38.00 (0.71)	40.50 (0.65)	40.00 (1.96)	39.75 (1.65)
12	36.40 (3.83)	37.00 (3.08)	39.20 (1.66)	41.20 (2.15)	40.80 (2.03)	40.60 (2.25)
14	35.50 (4.11)	39.00 (1.73)	38.00 (0.71)	38.00 (1.47)	37.50 (3.50)	38.75 (2.84)
16	32.00 (4.58)	39.67 (1.20)	40.00 (1.00)	38.00 (1.73)	38.00 (4.36)	38.33 (2.03)
18	33.67 (3.28)	40.33 (0.89)	39.00 (3.06)	39.25 (1.60)	39.33 (4.06)	38.00 (1.53)
20	34.25 (2.50)	39.00 (5.96)	40.60 (3.01)	41.60 (2.23)	41.50 (3.66)	40.60 (1.63)
22	34.25 (1.50)	40.50 (2.63)	37.25 (3.28)	40.75 (2.29)	41.50 (2.26)	39.50 (2.47)
24	25.33 (4.67)	37.00 (5.69)	33.33 (5.21)	38.67 (4.10)	40.33 (2.60)	38.00 (4.58)
26	24.67 (2.73)	33.67 (4.70)	30.00 (3.61)	38.67 (2.40)	38.00 (3.51)	34.00 (3.06)
28	16.00 (0.58)	24.67 (2.03)	31.00 (2.08)	36.33 (1.20)	38.33 (2.17)	38.00 (3.79)

Table 8. The red blood cell count ($\times 10^6/\mu\text{l}$) of mice infected with *Heligmosomoides bakeri* and treated with varied doses of *Duranta erecta* fruit extract [treatment was done on day 12].

Exptl. period (days)	Mean red blood cell count ($\times 10^6/\mu\text{l}$), with standard error in brackets					
	Group A (untreated control)	Group B (1000mg/k g extract)	Group C (500mg/kg extract)	Group D (250mg/k g extract)	Group E (Albendazole)	Group F (uninfected control)
0	9.58 (0.58)	9.64 (0.27)	9.83 (0.43)	10.15 (0.55)	9.63 (0.36)	9.42 (0.50)
4	6.89 (0.80)	4.71 (0.33)	6.83 (0.43)	5.51 (0.39)	4.95 (0.52)	9.77 (0.21)
8	5.63 (0.80)	5.26 (0.39)	4.88 (0.48)	6.59 (0.84)	6.39 (0.41)	9.63 (0.44)
12	4.04 (0.05)	3.89 (0.40)	3.51 (0.52)	3.43 (0.38)	3.24 (0.29)	9.54 (0.64)
16	4.24 (0.69)	5.16 (0.60)	5.13 (0.82)	4.22 (0.75)	4.69 (0.61)	10.40 (0.40)
20	3.98 (0.69)	5.49 (2.60)	6.38 (2.14)	6.72 (1.26)	7.42 (1.03)	10.18 (0.44)
24	2.67 (0.54)	4.98 (0.45)	6.00 (0.97)	6.62 (1.33)	8.01 (0.98)	9.81 (0.92)
28	2.00 (0.25)	3.63 (0.53)	4.57 (0.15)	5.36 (1.39)	8.84 (0.43)	9.37 (1.23)

Table 9. The total white blood cell count ($\times 10^3/\mu\text{l}$) of mice infected with *Heligmosomoides bakeri* and treated with varied doses of *Duranta erecta* fruit extract [treatment was done on day 12].

Exptl. period (days)	Mean total white blood cell count ($\times 10^3/\mu\text{l}$), with standard error in brackets					
	Group A (untreated control)	Group B (1000mg/ kg extract)	Group C (500mg/k g extract)	Group D (250mg/k g extract)	Group E (Albendazole)	Group F (uninfected control)
0	10.22 (0.77)	10.19 (1.16)	12.33 (1.59)	12.10 (1.29)	12.47 (1.58)	11.45 (0.37)
4	13.37 (1.41)	12.25 (0.81)	9.86 (1.21)	12.44 (0.42)	14.84 (0.68)	10.79 (1.03)
8	24.48 (2.09)	25.68 (1.61)	26.64 (1.93)	20.79 (2.22)	18.99 (2.60)	10.98 (1.35)
12	21.34 (6.01)	25.01 (4.08)	23.01 (5.73)	20.67 (2.49)	23.03 (3.97)	11.34 (0.62)
16	24.32 (3.00)	20.39 (1.97)	14.90 (0.58)	14.57 (0.89)	20.65 (1.74)	12.78 (0.88)
20	11.33 (2.13)	12.15 (3.86)	10.23 (1.20)	13.87 (2.12)	13.93 (3.63)	10.95 (1.12)
24	8.28 (2.13)	11.57 (3.39)	10.45 (1.44)	9.74 (1.42)	14.08 (0.66)	11.38 (1.59)
28	7.23 (1.89)	12.32 (1.44)	11.96 (0.94)	10.60 (0.38)	8.27 (0.77)	10.59 (1.74)

Table 10. Mean Worm count of mice infected with *Heligmosomoides bakeri* and treated with varied doses of *Duranta erecta* fruit extract

GROUPS		MEAN WORM COUNT $\pm$ SEM			DEPARASITIZATION
		(n = 3)			RATES
		MALE	FEMALE	TOTAL	
A	(Untreated Control)	32.8 $\pm$ 0.11	22.4 $\pm$ 1.24	55.2 $\pm$ 0.51	
B	(1000mg/kg extract)	24.8 $\pm$ 0.12	9.2 $\pm$ 0.42	34.5 $\pm$ 1.83	37.5%
C	(500mg/kg extract)	17.5 $\pm$ 1.31	31.3 $\pm$ 0.78	48.6 $\pm$ 2.10	11.96%
D	(250mg/kg extract)	14.6 $\pm$ 1.07	36.7 $\pm$ 0.11	51.3 $\pm$ 0.26	7.1%
E	(Albendazole)	2.2 $\pm$ 0.53	2.6 $\pm$ 0.12	4.8 $\pm$ 0.24	91.30%