In vivo and *in vitro* Anthelmintic Activity of *Balanites aegyptiaca* and *Artemisia herba Alba* on *Haemonchus contortus* of sheep

By

Rogia Osman Elhassan Albadawi

B.V. Sc 1996, M. V. Sc 2003,
Faculty of Veterinary Science
University of Khartoum

SUPERVISOR

Prof. Mohamed Magzoub Ahmed AlKan

Co-Supervisor,
Prof. Tigani Hassan ELamin

A thesis submitted to the University of Khartoum in fulfillment for the requirements of the degree of Doctor of Philosophy.

Department of Parasitology
Faculty of Veterinary Medicine
University of Khartoum

April 2010

DEDICATION

To the soul of my father,

To my Mother, Brothers and Sisters,

And to my Husband and Children

With my deep love.

Acknowledgements

First praise will be to Almighty ALLA for giving me health and strength to carry out this work. I wish to express my thanks, gratitude and indebtedness to my supervisor Prof. Mohamed Magzoub Ahmed Alkan, of the Department of Parasitology, Faculty of Veterinary Medicine, University of Khartoum, for his supervision, guidance, support and encouragement throughout this study.

I am also grateful to Dr. Shawgi, M. Hassan, of the Department of Parasitology, Faculty of Veterinary Medicine, University of Khartoum for his help and assistance in the statistical analysis and useful advice during the preparation of this thesis. Thanks are also extended to Dr. Mohamed Salih, Faculty of Agriculture, Soil department, for his help in the analytical structure of the study.

Thanks are due to all members of the department of Parasitology, Faculty of Veterinary Medicine University of Khartoum with special thanks and appreciation to Mr. Awadalla Abdelmoniem and Mrs. Entisar Abdella, for their assistance in different aspects during the period of my study. I would also like to thank Mr. Modather Elhassan the senior technician of phytochemistry lab, Institute of Medicinal and Aromatic Plants, for his help in the preparation of plant extraction. Thanks are also due to the staff of the electron microscope unit of the faculty of science, Ain Shams University, Cairo, Egypt. I would also like to thank Prof Ahmed Gameel for helping me in the histopathological studies and Mr. Yassin Jubara for processing histopathological samples. Also my thanks are

extended to Mr. Elsir for his help in microscopic photography and to all others who helped me during the experimental period.

My most special gratitude and indebtedness is given to my family specially my mother, for their patience, constant encouragement, understanding and unlimited support. I wish to offer my gratitude to my husband Adil for all the help, understanding, encouragement and unlimited financial support throughout my study. My work would not have been completed without the help and support of my family and my husband.

Last but not least my appreciation goes to my lovely children Raghad, Mohammed and Mahir for tolerating the situation of being separated from me most of the time during my work in Khartoum.

.

ABSTRACT

This study was carried out to evaluate *in vivo* and *in vitro* anthelmintic effects of the plants *Artemisia herba alba* and *Balanites aegyptiaca* against the nematode of sheep *Haemonchus contortus*.

For *in vivo* study, 28 lambs (4-5 months old) of Hamari type were used. They were fed on dura and dry groundnut hay. During this period, they were given two doses of each of anthelmintics, antibiotics, and anticoccidial drugs to treat them from any possible infections. They were randomly divided into 7 groups of 4 lambs each. Six groups were orally infected with a single dose of 3000 third larval stage of *H. contortus* (L_3). Two of these groups were given orally 3g/kg bodyweight of *A. herba alba* water extract (one group was given the extract one day before infection and the second group after 25 days from infection). Similarly, the 3rd and 4th groups were given 9g/kg of fruit mesocarp of *B. aegyptiaca* (one group was given one day before infection and the second group after25 days from infection). The fifth group was treated with albendazole (5mg/kg b wt) after 25 days from infection. The sixth group was infected but not treated and left as a control group. The 7th group was kept as uninfected untreated control. Faecal and blood samples were weekly collected for egg count and haematological values. The infected and treated lambs were slaughtered after 6 weeks from infection and the numbers of adult worms in the abomasi were counted.

All infected animals showed a significant decrease in Hb concentration, PCV, RBCs count and liveweight gain compared with the uninfected control. Animals treated with *A. herba alba* and *B. eagyptiaca* before infection showed slight decrease in egg per gram (epg) and total worm burden, compared with the infected untreated control. Treatment

after infection with *B. aegyptiaca* showed anthelmintic efficacy of 69.9% and maximum reduction of 69.6% in epg of faeces on day 14 post treatment, compared with 62.5% and 61%, respectively for *A. herba alba* and 97.4% and 98.5%, respectively for albendazole.

Lambs treated with *A. herba alba* and *B. aegyptiaca* before infection, showed no significant increase in Hb, PCV and RBCs count. However, animals treated after infection, exhibited significant increase in Hb, PCV and RBCs count two weeks post treatment. No significant differences were observed in leukocyte count, eosinophils, lymphocytes, neutrophils and liveweight gain in animals treated before or after infection compared with the infected untreated control. No evidence of toxicity of *A. herba alba* and *B. aegyptiaca* was observed during or after infection.

For *in vitro* study, aqueous and methanolic extracts of *A. herba alba* and *B. aegyptiaca* at concentrations of 5, 10, 15 and 50 mg/ml were conducted on adults and larvae (L_3) of *H. contortus*. The extracts of the two plants exhibited anthelmintic effects on the adults. The dose rate of 50 mg/ml showed a significant anthelmintic effect in *A. herba alba* with greater activity of methanolic extract (100% mortality after 2 hours) than the aqueous extract (90% mortality after 6 hours). However, the aqueous extract of *B. aegyptiaca* had a higher anthelmintic activity (100% mortality after 6 hours at 50mg/ml) compared with the methanolic extract (70% mortality after 6 hours at 50mg/ml). The aqueous and methanolic extracts of *A. herba alba* and *B. aegyptiaca* as well as albendazole had no effect on the larvae.

The ultrastrucure of some of the nonmotile worms after treatments with the plants was examined under electron microscope. Damage was observed in the tegumental muscles, digestive tract and reproductive system. The muscle fibers were hypertrophied, the microvilli of the

intestines were spaced, some were lost, others were irregular and the reminders were reduced in size. The vetelline glands of the reproductive system showed numerous droplets and vaculations. The mitochondria became elongated; some were small and others were swollen.

It is concluded that *B. aegyptiaca* and *A. herba alba* had an *in vivo* and *in vitro* anthelmintic effects on the adult *H. contortus*, but they lacked prophylactic activities when given before infection.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF PLATES

Introduction

Sudan is a tropical country with a land area of one million square miles, and has a population of 40.3 million inhabitants. The main economic activity displayed by the inhabitants is agriculture and animal production. Sudan is considered as one of the most important countries in Africa and the Middle East in as far as animal production and export of livestock is concerned. The most recent animal census estimated in livestock population is 44.8 million heads of sheep, 35.8 million heads of cattle, 3 million heads of camels and 37.3 million head of goats (AOAD, 2001).

The sheep population sector plays a great role in the economy of the country and is kept mainly for meat production. The Sudan is capable of producing sufficient amounts of mutton for local consumption and for export as well.

The Diseases caused by helminth parasites in livestock continue to be major productivity constraints, especially in small ruminants in tropical and subtropical countries (Perry *et al*., 2002). Helminthiasis plays a crucial role in small ruminant's production leading to enormous economic losses. It causes loss of production through mortality, weight loss, reduced milk and meat production (Ketzis *et al*., 2002; Githiori *et al*., 2003). Haemonchosis, as an important helminthic disease which is caused by the highly pathogenic nematode parasite of small ruminants *Haemonchs contortus*, causes acute diseases and high mortality (Gagood and Eisa, 1968; Allonby and Urquart, 1975; Sulieman, 1989). Consequently, there is an urgent need to control the infections caused by *H. contortus* in small ruminants.

Helminth Control in domestic animals is widely based on the use of anthelmintic drugs in combination with grazing management. In the Sudan, the method of controlling internal parasites which relies on rotational grazing (Barger, 1999) is limited and so it is difficult to be practiced in this country. The livestock are mostly raised under nomadic conditions with traditional methods of management and natural grazing. Therefore, the control of helminth infections in animals to alleviate production losses relies totally on the use of anthelmintics; in the absence of sustainable control programs that can reduce the dependence on chemical control agents. However, the current efficacy of these drugs has been reduced, because of resistant nematode strains (Bartley *et al*., 2003; Jabbar *et al*., 2006; Artho *et al*., 2007). The misuse in the application of chemical anthelmintics and some times poor quality drugs (Githiori, *et al*., 2004) led to a development of high level of multiple anthelmintic resistance in many parts of the developing countries (Waller, 1997). Furthermore, the high cost of these drugs together with the residual concern in food animals and the risk of environmental pollution have awakened interest in medicinal plants as an alternative source of anthelmintic drugs (Pessoa *et al*., 2002; Hordegen *et al*., 2003; Githiori, 2004; Bizimenyera *et al*., 2006). The combination of these factors has stimulated the search for alternative control strategies. Amongst these strategies, is the use of traditional plant remedies. In Sudan, the use of plants as anthelmintics by smallholder farmers is practiced in different ways. Plants have been used from ancient times to cure diseases of man and animals. This is particularly so in regions of the country where modern anthelmintics may not be available or, when available, but are too expensive for these farmers. The use of plants as anthelmintics could therefore be a potential alternative for smallholder farmers and pastoralists to control helminthes.

The objectives of this study are:

1- To determine *in- vivo* the curative, prophylactic and suppressant effects of albendazole and the medicinal plants *Balanites eagyptiaca* and *Artemisia herba alba* against *H. contortus*.

2- To determine *in- vitro* the effects of albendazole and the medicinal plants *B. eagyptiaca* and *A. herba alba* on the motility of the adult and third larval stages of *H. contortus*.

3- To study *in-vitro* the histological structure under Electron microscope of normal and treated adult *H. contortus* with albendazole and the medicinal plants *B. eagyptiaca* and *A. herba alba* to determine the mechanism of action of the drug and the medicinal plants.

CHAPTER ONE
LITERATURE REVIEW

1.1 Taxonomy and morphology of *Haemonchus contortus*:

Haemonchus contortus (Rudalphi, 1803) is the species which is most commonly found in sheep and goats. Its taxonomy was given by Chitwood (1969), Soulsby (1982) and Taylor *et al.,* (2007) as follows:

Phylum	Nemathelminthes	Schneider (1873)
Class	Nematoda	Rudolphi (1808)
Subclass	Secernentea	Doughert (1958)
Order	Strongylida	Molin (1961)
Superfamily	Trichostrongloidea	Cram (1927)
Family	Trichostrongylidae	Liper (1912)
Genus	*Haemonchus*	Cobb (1898)
Species	*contortus*	Rudolphi (1808)

Gibbson (1979) has reported that the genus has nine valid species. Among these is *Haemonchus contortus* which dwells in the abomasum of sheep, goats and to a lesser extent bovine. *Haemonchus placei*, and *H. longstipes* which are found in cattle, and camels respectively also develop in sheep and cause clinical haemonchosis but of less severity than that caused by *H. contortus* (El Bihari *et al*., 1984; Blood and Radostitis, 1989; Le Jambre, 1995).

The nematode *Haemonchus* possesses a small buccal cavity with a slender tooth or lancet; cervical papillae are prominent, the bursa is large especially the lateral lobes while the dorsal lobe is small, asymmetrical and is supported by a Y-shaped dorsal ray. Males are 10-20 mm long while

females are 18-30 mm long. The male has an even reddish colour, while in the female; the white ovaries are spirally wound around the red intestines, producing the appearance of a barber's pole. The vulva of the female is usually covered with alinguiform process (vulval flaps). The eggs measure 70-85 µ by 41-48 µ and contain an embryo which is divided into 16-32 cells (Soulsby, 1986; Taylor *et al*., 2007).

1.2 Life cycle of *Haemonchus contortus*:

The life cycle of *Haemonchus contortus* has been studied perhaps more extensively than any other sheep nematode. It was described by Ransom (1906) and Veglia (1915) and it has a direct life cycle. The parasite alternates in its development between the free living generation (first, second, and third larval stages) and the parasitic one (fourth and fifth larval stages and mature worms). Adult worms lay strongyle type of eggs into the lumen of the gastrointestinal tract. The female of *H. contortus* lays about 500-15000 eggs per day (Hansen and Perry, 1994). There are many factors which affect the fecundity of *H. contortus* female and faecal egg excretion, such as the number of worm burden, host immunity, the physiology of the host (e.g: parturition) and the age of the worms. At the beginning of egg production, the female worm lays a small number of eggs per day. This will then increase rapidly to reach a peak at 25 to 30 days post infection. The eggs pass in faeces and under sufficient moisture and warmth, embryonate and hatch in faeces through three successive larval stages (L_1, L_2, and L_3). The first and the second stage larvae (L_1 and L_2) had a rabditiform oesophagus and they live freely, feeding on the faecal microflora and develop to the infective third stage larvae (L_3) within 6 to 7 days at the optimum temperature of 22-26°C (Dunn, 1978). The third larval stage fails to cast the loose cuticle of the previous stage and therefore,

remains ensheathed in a non-feeding form. It disperses from the faecal mass onto herbage, where when moisture is adequate, it survives until it invades the host. It has well survival ability in different conditions because of the cuticle sheath. The larvae migrate horizontally and vertically according to changes in temperature, moisture and humidity during the day time. They can also migrate up and down the plates of grass according to the amount of moisture. For example, when the dew is on the grass or there is a rainfall, the larvae migrate to the top of herbage, but following evaporation of water the larvae will migrate down the herbage and even into the soil (Magzoub *et al*., 1990; Hansen and Perry, 1994).The larvae are usually found at the top of grasses during early morning and late afternoon. This behavior is a survival mechanism for the parasite before finding a suitable host.

Infection of the animal occurs by the ingestion of food or water contaminated with the third infective stage larvae (L_3). When the larvae (L_3) reach the rumen, they exsheath in response to physicochemical stimuli principally CO_2 within the gut (Rogers and Sommerville, 1968). Exsheathment involves the release of exsheathing fluid, which contains enzymes capable of attacking the sheath (Rogers and Sommerville, 1982). Then the larva by its own movement escapes from the sheath. Forth stage larvae then migrate into the abomasal mucosa and penetrate between the gastric epithelial cells and develop into fifth stage larvae within four days. They may either become hypobiotic or develop to maturity and start egg laying 15-21 days after infection depending on the seasonal condition (Hunter and Mackenzie, 1982). The prepatent period of *H. contortus* may reach 21 days. It takes different values depending on the environmental condition, such as host immunity (Rahman and Collins, 1990).

1.3 Epidemiology:

Many factors i.e external (environmental) and internal (host) affect this pattern of development and hence the epidemiology of the infection. External factors like weather, climate and bionomics of ruminant nematode larvae on pasture were reviewed by Le- Jambre (1981) and Taylor *et al*. (2007). They pointed out that, the two most important components of the external environment are temperature and humidity.

1.3.1 Temperature and Humidity:

The optimal temperature for the development of the maximum number of larvae is generally in the range of 18-26°C. At higher temperatures, the development is faster and the larvae are hyperactive, thus depleting their lipid reserves. The mortality rate then rises, so that few will survive to L_3. As the temperature falls the process slows, and the development below 10°C from egg to L_3 usually does not take place (Taylor *et al*., 2007). Generally, cool, dry weather prolongs larval survival, and hot, wet weather shortens it. This is attributable to the fact that infective larvae do not feed, and must survive on stored energy. Low temperatures and dry conditions prevent active movements by the larvae and thus minimize their energy expenditure. Larval survival times on pasture range from few weeks in the wet tropics (Banks *et al*., 1990; and Barger *et al*., 1994) to well over a year in temperate climates (Barger *et al*., 1984).

The optimum relative humidity for the development of larval stages is 100%, although some development can occur down to 80% relative humidity. However, in dry weather where the ambient humidity is low, the microclimate in faeces or at the soil surface may be sufficiently humid to permit continuation of larval development (Urquhart *et al.,* 1987 and Taylor *et al*., 2007). On the other hand, Okon and Enyerhi (1977)

pointed out that the dry season is unfavorable for the development and survival of free-living stages of *H. contortus*. This was attributed by Chiejina *et al.*, (1989) to the rapid drying out of the faecal pellets during hot dry weather.

Two internal factors are considered to play an important role in the epidemiology of *H. contortus* infection. Those are the arrested development and selfcure mechanism.

1.3.2 Arrested development of *Haemonchus contortus*:

Arrested development is one of the factors affecting the epidemiology and immunology of haemonchosis. It is defined as the temporary cessation in the development of a nematode at a precise point in its parasitic development (Michel, 1974). The factors governing arrested development of gastro-intestinal nematode parasites are as yet poorly determined. It was initially thought that hypobiosis in *H. contortus* was induced by host resistance to worm infection (Michel, 1974; Waller and Thomas, 1975; Adams, 1983), but more studies have shown that environmental changes may play a more significant role (Gibbs, 1986). Environmental factors were discussed by some authors who considered the phenomenon as a response to these factors acting upon the infective larvae in a similar manner to diapause in insects (Armour *et al*., 1969 a, b; Armour and Bruce, 1974; Armour, 1978). In Senegal, Vercruysse (1985) concluded that *Haemonchus* sp. survives the dry season as hypobiotic larvae as well as adults. Significant numbers of inhibited larvae appeared early in the dry season (November) and they represent 49% of the total in December. A similar proportion of inhibited larvae were present until April and after that the number of inhibited larvae declined abruptly (Vercruysse, 1985). Gatongi *et al*. (1998) reported that both adult worms and hypobiotic

larvae were found in proportions that varied with seasons. Statistically, a higher proportion of hypobiotic larvae was found during the dry months than during the wet months, an indication showing that hypobiosis was an important feature in the survival of *H. contortus* during the dry months. Because the infective stage of this nematode is highly susceptible to winter conditions (Kates, 1950; Crofton, 1963), the nematode survives the winter in the hypobiotic form and resumes development when climatic conditions become favourable for transmission (Anderson, 1972; Reid and Armour, 1972; Michel, 1974). Other workers assigned the phenomenon to factors such as blood group and the breed of sheep (Knight *et al*., 1973; Soulsby, 1982). In connection with immunity, Soulsby (1966) reported that arrested development was generally ascribed to high level of resistance in the host. On the other hand, Pradhan and Johnstone (1972) noticed that when animals become infected with maximum numbers of L_3, arrested development occurs. Also Dineen *et al.* (1965) showed that small daily doses of L_3 for a prolonged time lead to greater proportion of arrestment than those that were given on a single occasion.

The epidemiological importance of hypobiosis, is that the resumption of development of hypobiotic larvae usually occurs when conditions are optimal for free living development and so results in an increased contamination of the environment (Urquhart *et al.,* 1996).

1.3.3 Self-cure phenomenon:

Self-cure phenomenon is defined as an abrupt rejection of established adult worms in suitably infected and sensitized sheep by a challenge dose of infective larvae (Stewart, 1955). It has a great epidemiological importance in the control of *Haemonchus* infection (Gordon, 1948). It was originally described by Stoll (1929) who noticed

that sheep infected with *H. contortus* when allowed to graze in contaminated pasture showed suppression of egg production that was often accompanied by elimination of adult worms. Moreover, Stewart (1950, 1953) showed that the phenomenon was almost certainly due to massive infection and he provided an evidence of an allergic response that was involved in the expulsion of worms.

The investigation of self-cure reaction has been hindered by the lack of a predictable system for reproducing the phenomenon (Adams, 1983). Many workers tried to elucidate the possible cause of the phenomenon and they gave different ideas. Radhakrishnan (1972) suggested a relationship between blood haemoglobin type and the ability to mount a self-cure reaction. Allonby and Dargie (1973) suggested that climatic or pastural changes are themselves, sufficient stimuli for self-cure mechanism. Allonby and Urquhart (1973) in Kenya showed that under field conditions self-cure mechanism can occur and the phenomenon is apparently non-immunological but it is a flock phenomenon that occurs simultaneously on one occasion in sheep of all ages.

1.4 Pathogenesis:

The pathogenecity of *Haemonchus* infection is mainly related to blood loss. The fourth stage larvae and the adult worms are bloodsuckers. They pass large amounts of blood through their digestive tract from affected sheep causing loss of all blood elements including red cells and plasma proteins. The average blood loss has been calculated as 0.05ml/parasite/day. Thus anaemia and hypoproteinaemia result from infection (Clark *et al*., 1962; Ahmed and Ansari, 1989; Albers *et al*., 1990; Ahmed *et al*., 1990).

The pathogenisis of haemonchosis depends upon many factors that control the dynamic and the density of the pathological changes of the disease. These factors including, the dose of infection, number of total worm burden, the nutritional status of the host, immunity of the host and the age of the animal (Soulsby, 1982; Hansen and Pery,1994; Urquhart *et al*., 1996). Soulsby (1982) reported three forms of haemonchosis depending on the magnitude of the worm burden. Animal harboring ≤ 1000 worms showed chronic haemonchosis, animal with 1000-10000 developed acute haemonchosis and animal with more than 10000 showed para- acute disease. Also Dargie (1975) reviewed the pathology and the significance of haemonchosis in sheep. He considered three phases of haemonchosis that might occur; in the first, sheep may lose large amounts of blood in the first three weeks of infection. Because the erythropoietic system requires time to adjust and to increase its output of blood, then an outbreak of acute haemonchosis may occur. In the second phase (from 1-2 months) although there may still be a sustainable loss of red blood cells, packed cell volume (PCV) values may not decrease further as the host is able to compensate by increasing erythropoiesis. In the final phase PCV may decline rapidly as the erythropoietic system becomes depleted due to iron deficiency and possibly to reduction in the availability of amino acids. Moreover, Abbott *et al*., (1986); AlQuaisy *et al*. (1987); Ahmed and Ansari (1989); Mottelib *et al*. (1992) and Ghulam-Rasool *et al*. (1995) reported that haemonchosis in sheep significantly decreases body weight gains, haemoglobin concentration, PCV values and total erythrocytes count. These changes are significantly correlated with high parasitic burden and changes in WBCs count which are consistent but showed a tendency to decrease (Ahmed and Ansari, 1989). The examination of blood and faecal samples from four, one-year old sheep (given $4x10^3$, $8x10^3$ and $12x10^3$ infective third stage

larvae of *H. contortus*) revealed a degree of anaemia due to haemonchosis which is positively correlated with the number of eggs per gram (epg) of faeces. No significant difference in body weight gain was found between animals given different doses of larvae, but all infected animals significantly lost bodyweight as compared to the controls (Khan *et al*., 1988).

Reduction in serum proteins and albumins has been described in sheep infected with *H. contortus* (Abbott *et al*., 1986 ; Rahman and Collins, 1990). Ahmad *et al*., (1990) infected experimentally three groups of lambs with $4x10^3$, $8x10^3$ and $12x10^3$ third infective stage larvae of *H. contortus* and found that, total serum proteins, albumins, and globulin ratios showed a corresponding and proportional decrease. Observation of Pradhan and Johnstone (1972) in lambs infected either weakly or daily with *H. contortus* showed a reduction in sodium but a rise in potassium concentration in erythrocytes. Abakar (1996) studied the changes in serum constituents of lambs experimentally infected with *H. contortus*. He reported a decrease of serum total proteins, albumins and globulin values when compared with uninfected lambs. Phosphorus level failed to show consistent pattern of change throughout the observation period. Serum iron, sodium and calcium values were significantly declined ($P < 0.05$) while mean serum potassium concentration showed no significant differences between infected and uninfected animals.

Gross pathological lesions were shown to occur due to migration of the larvae of *H. contortus* into pits of the gastric glands in the abomasal wall and the physical injury caused to the mucosa by the attachment of the adult worms causing abomastitis (Blood and Rodostitis, 1989). Alzubaidy *et al*. (1987) demonstrated experimentally the gross pathological changes shown from a single infection with 500 *H. contortus* larvae per kg body

weight of sheep and goats. They found that at day 4 post infection (pi), the gastric mucosa especially the cardiac region was congested and showed white spots. At day 11 (pi), there was congestion, haemorrhage, oedema and thickening ridges of the infected gastric mucosa. Oedema and congestion of the parasitized abomasal mucosa and the cellular inflammatory reaction were more marked by day 15 pi. At day 21 pi, gross examination of the infected abomasi revealed only the presence of granular uneven mucosal surfaces in addition to the presence of slight congestion and minute petechial haemorrhages scattered between whitish large nodules (Alzubaidy *et al*., 1987). The histopathological changes in the abomasum of sheep during infection with *H. contortus* were observed by Charleston (1965) after experimental infection of lambs. The changes included abomasal mucosa hypertrophy together with marked mononuclear cells and eosinophil infiltrations. Similar pathological changes were reported by malczewski (1970), who suggested that loss of a proportion of the young adult worm population around 10-14 days after infection was related to a heavy infiltration of the abomasal mucosal membrane and aggregation of eosinophil cells after re-infection with *H. contortus*.

1.5 Diagnosis of ovine Haemonchosis:

Parasitological techniques used for diagnosis depend mainly on faecal examination to detect the presence of worms, eggs or larvae. However, there are several methods to be used for preparing faeces for microscopic examination; direct smears are the most commonly used, although the floatation method is usually necessary to detect low grade infections (Soulsby, 1986). Repeated detection of eggs of trichostrongylid worms in faeces of animals might not be diagnostically significant and frequently larvae culture becomes necessary for generic identification

(Roberts and O'sullivan, 1950). All these methods depend on egg shedding by the adult worms or on postmortem examination. On the other hand the diagnosis of haemonchosis is usually based upon evaluation of clinical signs and faecal examinations, which have their limitations. Clinical signs usually become apparent when the infection is heavy. Eggs are found in feces after the prepatent period of approximately 3–4 weeks in which infections were firmly established and damage has already been done. So a reliable serological assay such as ELISA, which enables detection of early infection and subclinical infection, is needed (Almazan *et al*., 2001). However, serodiagnosis of parasitic disease, for the start, was faced or hindered with some constraints as it should ideally be sensitive and specific not only for the parasite species but also for each stage of a given parasite (Walls and Schantz, 1986; Buijs and Buitenberge, 1987). That was due to the complexity of helminth antigens and the shared cross reacting antigens hence, highly purified parasite extracts should be employed in order to improve specificity (Weiss *et al*., 1982). An example of serological diagnostic methods is immunoassay, such as radioimmunoassay (RIA) and Enzyme-linked immunosorbent Assay (ELISA). The two tests have become accurate means in diagnosis of parasitic diseases because of their high sensitivity, specificity, rapidity and reproducibility. Both assays have been utilized widely to detect parasite antigens or parasite specific host antibodies in host body fluids (Voller and Desavigny, (1981); Schalling *et al*., 1995). Nematode excretory/secretory (ES) products were shown to have important biological function (Hotez *et al*., 1990; McKerrow *et al*., 1990; Richer *et al*., 1992); ES products were used in diagnostic antigen, which had good sensitivity and specificity (Gamble *et al*., 1988; Mahannop *et al*., 1992; Schallig *et al*., 1995; Choi *et al*., 2003). Xiaojun Li *et al*. (2007) demonstrated that the prokaryotic-expressed hexahistidyl peptide

fusion protein (His-ES24) might be a useful diagnostic reagent for epidemiological studies of *H. contortus* in sheep. Harmon *et al*. (2007) demonstrated the usefulness of a quantitative real-time PCR (QPCR) for amplification and quantification of trichostrongyle eggs, and identified potential limitations, which may be addressed through multiplex assays or the addition of a standard: exogenous DNA target.

1.6 Control of *Haemonchus contortus* infection:

Control of infection with gastrointestinal nematodes is a perennial and increasingly difficult problem. The main methods for the control of GI nematode parasites are by treatment with synthetic anthelmintics (Waller 1986; Kloosterman 1992). However, alternative strategies have also been examined for the sustainable control of GI nematode parasites. A brief overview of the various methods of control is described below.

1.6.1 Control with Anthelmintics:

Haemonchus contortus has been ranked as the most important parasite of small ruminants in all regions across the tropics/subtropics (fabiyi *et al*., 1987). Fortunately, this species is susceptible to a greater range of anthelmintic groups than other nematodes (waller, 1997). Anthelmintic treatment continues to be the most commonly used method to control infection of gastrointestinal nematodes. There are only three broad-spectrum anthelmintic groups available for treatment of grazing animals for the control of nematodes. Group 1, the benzimidazoles (BZ), group 2, the imidazothiazoles (levamisole, and hydropyrimidines (pyrantel/morantel), and group 3, the macrocyclic lactones (avermectins and milbemycins,ML), have different mechanisms of action. The salicylanilides and nitrophenols are used as narrow spectrum anthelmintics for the control of *Haemonchus*

contortus in sheep, and in some countries organophosphates are still marketed. No new anthelmintics with different modes of action are expected on the market in the near future (Coles *et al*., 2006). Among the Benzimidazole group (BZs), Thiabendazole, Oxfendazole, Cambendazole, fenbendazole and Albendazole were reported to have a high broad spectrum and a varying activity against gastrointestinal nematodes and *Haemonchus* infections (Colglazier *et al.,* 1972; Ross, 1975; Averkin *et al*., 1975; Kirsch and Duwel, 1975; Williamson, 1995). The mode of action of BZs is by interference with polymerization of microtubules (Harder, 2002). These drugs induce binding to the protein tubulin of the parasite, therefore causing death by starvation (Roos, 1997). The tetrahydropyrimidines and imidazothiazoles group (levamisole and pyrantel– morantel), were reported to be highly effective against both mature and immature gastrointestinal nematodes (Thiempont *et al*., 1966). These drugs affect acetylcholine neuro-transmission by interfering with nicotinic acetylcholine receptors (Roos, 1997; Harder, 2002). The macrocyclic lactones (MLs) or avermectins/milbemycins groups are thought to interact with chloride channels on helminth gammaaminobutyric acid (GABA) receptor complexes, and also inhibit pharyngeal pumping (and hence feeding), motility and fecundity in susceptible nematodes, resulting in paralysis and ultimately elimination from the host (Harder, 2002; Yates, *et al*., 2003). Phenothiazine (Thiodiphenylamine) has been successfully used for more than forty years in the treatment of parasitic gastroenteritis in domestic animals. It has a high efficacy against abomasal worms, but has no effect on the larval stages (Boughton, 1940; Forsyth *et al*., 1961).The Organophosphorus compounds in general had their origin as pesticides and only subsequently found as anthelmintics (Meyer *et al*., 1977).

There are other anthelmintics referred to as narrow spectrum compounds, which have activity against fewer species of parasites and/or lack high levels of efficacy against all stages of the parasites (Bowman, *et al*., 2003). Examples of these anthelmintics include naphthalophos, salicylanilides and substituted phenols (closantel, oxyclozanide and nitroxynil), and triclabendazole.

1.6.1.1 Anthelmintic resistance:

One of the main problems facing the use of chemotherapy of helminthes infection is anthelmentic resistance in trichostrongylid nematodes, which has been reviewed by many workers (Le Jambre, 1978; Prichard *et al*., 1980; Banks 1988; Waruiru *et al*., 1998; Waller *et al*., 1996). It has been reported that some strains of *H. contortus* have been shown to have multiple resistance to benzimidazole, levamisole, morantel and naphthalphos (Jambre, *et al*., 1976). Berger (1975) reported the parbendazole resistance of *H. contortus* after six years of exclusive use of the anthelmentics on one farm. Resistance of *H. contortus* to rafoxanide , closantel and ivermectin was identified by Van-Wyk and Malan (1988) and Gill and Lacy (1998). High levels of resistance (exceeding 80% of farms) to both the benzimidazole and the levamisole/morantel groups, and their combinations, were recorded in all countries of South America, (Nari *et al*., 1996; Maciel *et al*., 1996). Recent surveys showed that combined resistance to the benzimidazoles and levamisole occurred on approximately one-third of commercial farms in Fiji and resistance to ivermectin was also emerging (Manueli, 1996). Further survey work in Malaysia (Dorny *et al*., 1994) has shown high levels of resistance, particularly to the benzimidazoles, in parasites of small ruminants, and multiple resistance involving all broad-spectrum anthelmintic groups, as well as closantel has

recently been reported (Sivaraj *et al*., 1994). Anthelmintic resistance is also increasing on the sub-continent of India (Gill, 1993). In Kenya, resistance is widespread, particularly to the benzimidazoles (Wanyangu *et al*., 1996). Recent surveys in South Africa show that around 90% of sheep farms have parasite strains resistant to compounds from at least one anthelmintic group and approximately 40% of the farms now have to confront the problem of multiple anthelmintic resistances (van Wyk *et al*., 1998). In Ethiopia Eguale *et al*. (2009) reported that *H. contortus* has developed resistance to albendazole. The prevalence of benzimidazole resistant nematodes in dairy goats in France ranged from 70 to 100% of the farms (Chartier *et al*., 1998, 2001). The first documented case of ivermectin resistance of a gastrointestinal nematode of small ruminants in the Netherlands was reported by Eysker *et al*. (2006). They showed that *H. contortus* appears to be resistant to oxfendazole and *T*eladorsagia *circumcinta* appears to be resistant to oxfendazole and ivermectin. Artho *et al*. (2007) indicated that ivermectin resistance is widespread in Swiss small ruminant farms keeping Boer goats and Dorper sheep. The findings of Howell *et al*. (2008) provided strong evidence that anthelmintic resistance is a serious problem on small ruminant farms throughout the southeastern United States.

1.6.2 Vaccination:

Enormous research efforts and funding have been expended on attempts to develop reliable and cheap vaccines which provide reasonable levels and length of protection against the important parasites in grazing sheep (Drudge *et al*., 1957). However, the difficulties of producing live larval vaccines are often cited as a reason why this line of research should not be pursued.

Administration of irradiated larvae can confer high levels of resistance in older sheep (Jarrett *et al*., 1959; Mulligan *et al*., 1961). However this method does not induce protection in young lambs which are highly susceptible to *H. contortus* (Mulligan *et al*., 1961). It has been shown that the ability of lambs to develop immunity to the abomasal nematode *H. contortus* is apparently insignificant. This was confirmed by laboratory investigations which indicated that previous infection with *H. contortus* in young lambs 2-5 months old confers no protection against a subsequent challenge (Manton *et al*., 1962; Urquhart *et al*., 1966). In contrast, immunization of sheep reared worm-free until at least 7 months of age either with normal or irradiated larvae conferred a very high degree of protection to a subsequent challenge (Jarrett *et al*., 1961; Manton *et al*., 1962). There are a number of reports of failure to induce effective immunity with irradiated *H. contortus* vaccine or viable infection until lambs are 6 months old (Urquhart *et al*., 1966). Conversely, several reports have indicated that 4- month old lambs can be effectively vaccinated with irradiated *Trichostrongylus colubriformis* vaccines (Windon *et al*., 1979). Current mathematical models on worm control have shown that a vaccine yielding 60% protection in 80% of the herd, or flock, would be a highly valuable control tool (Barnes, *et al*., 1995). The only commercially successful vaccine against a parasitic nematode is the attenuated larval vaccine described by Peacock and Pointer (1980) for use against the bovine lungworm *Dictyocaulus viviparous*. Following the success of the irradiated larval vaccine against bovine lungworm, *Dictyocaulus viviparus,* and a similar approach using irradiated *H. contortus* L3 was found to consistently offer good protection in sheep older than six months (Gray, 1997). The case for seeking to develop molecular vaccines against ruminant gastrointestinal parasites has been summarized by Emery and Wagland (1991). Charles, *et*

al. (2007) suggested that HC58 DNA vaccine conferred some protection against *H. contortus* infection in goats.

Current research on helminth vaccines has generally concentrated on the production of synthetic or recombinant vaccines using either natural or hidden (concealed) antigens (Schallig, *et al*., 1997; Smith *et al*., 2003).

Strategies for vaccine development fall into 2 broad categories: (i) hidden antigens, which are generally, but not always extracted from the gastrointestinal tract of the adult parasite and are not seen by the host's immune system during the course of infection. Vaccination with these gut antigens provides solely antibody –mediated immunity; (ii) natural antigens which are seen by the hosts immune system during the course of infection, are usually derived from the early infective larval stages of the parasite (L3s), and protection is believed to involve both antibody and cellular responses (Newton, 1995).

Many techniques such as oral administration of X-irradiated larvae of *H. contortus*, and injection of metabolic products released by *H. contortus* third and fourth stages larvae cultured *in vitro* were tried or shown to be useful in vaccination of sheep against *H. contortus.* Also parenteral injection of relatively small amounts of extracts of third and fourth stage larvae and killed *H. contortus* as an antigen in sheep was found to induce a protective immunity against infection (Murry 1973; Smith 1977; Murry *et al*., 1979; Kabagambe 2000). On the other hand, Boisvenue *et al*., (1991) suggested that the fibrinogen degrading proteins of infected sheep with *H. contortus* have a protective role in vaccination of sheep against *H. contortus*. Although the mechanism by which sheep are protected is not known, it may be due to local production of 1gA in the abomasal mucus, coincidental or additional to the circulating antibody responses (Smith and Christie, 1979). There also may be a functional cell

mediated response and a vaccine that would stimulate both types of immune responses (humoral and cellular) will be the most effective in combating haemonchosis (Duncan *et al*., 1978).

1.6.3 Genetic aspects of *H. contortus* infection:

Resistance has been described as the ability of the host to prevent or limit the establishment or development of infection. Considerable research over decades has been undertaken in many sheep rearing countries to identify breeds that have natural resistance to GI nematode parasite infections (Woolaston and Baker, 1996; Raadsma *et al*., 1998). Series of studies have shown that breeding for gastrointestinal nematode resistance is an alternative method for controlling helminth parasites that lower sheep and goat production (Piper, 1987; Gray, 1987; Gray *et al*., 1987; Schmidt *et al*., 1994). Various reports indicated the presence of substantial variations among sheep and goat breeds in resistance to gastrointestinal parasites particularly *H. contortus* (Zajac *et al*., 1990; Gamble and Zajac, 1992; Matika *et al*., 2003; Mugambi *et al.*, 2005). Albers *et al*. (1987) reported that many genes appeared to be involved in the control of resistance, and they concluded that selection for polygenitically controlled resistance would lead to increase productivity of infected animals. In U.S.A, Scrivner (1964) showed that Targhee and Panama breeds have shown to be more resistant to infection with *H. contortus* than Hampshire, Suffolk and Rambouillet breeds. Furthermore, Florida native lambs reported to be more resistant than Rambouillet lambs to *H. contortus* infection (Bradley *et al*., 1973). Zajac *et al*. (1990) observed that feacal egg counts were highest in Rambouillet and heamatological values and were more severely affected than in Florida Native and St Croix lambs. In Brazil Costa *et al*. (2000) reported that The Anglo-Nubian breed had higher PCV and Hb and Bhuj breed had intermediate values, however Caninde breed had lowest values.

In France, during a study which was conducted in a flock of 290 ewes of Romanov (R), Merino (M) and Romanov X Merino (RM), significant higher resistance was found to occur in R than RM and M ewes for the strongyles, *Teladorsagia circumcincta, Oesophagostomum venulosum* and *Nematodirus spp.* (Gruner *et al*., 1992). In Yugoslavia, adult Ciyaja and Merino prekos sheep were more resistant to infection with *H. contortus* than Merion Karkas sheep (Cvetkovic *et al*., 1978). In Indonesia, Subandriyo *et al*. (1996) and Romjali *et al*. (1996) showed variation in susceptibility to *H. contortus* infection between Javanese fat tail and Sumatra breeds. Australian workers have shown that selection for polygenically controlled resistance would lead to substantial progress and increase production of animal infected with *H. contortus* (Riffkin and Dobson, 1979 and Albers *et al*., 1987). In East Africa Preston and Allonby (1979) reported higher level of resistance of Red Masai sheep to *H. contortus* than Dorper sheep. Also In Kenya, it has been demonstrated that the local Red Maasai (RM) sheep and small East African goats are more resistant than the Dorper sheep and Galla goats, respectively (Shavulimo *et al*., 1988; Mugambi *et al*., 1997; Baker, 1998; Baker *et al*., (1999); Baker *et al*., 2003). Gebrekiro (1990) compared four breeds of indigenous Ethiopian sheep for their resistance to *H. contortus* at Awasa in southern Ethiopia. He observed that the Blackhead Somali were the most susceptible to endoparasites, while the Aris were the most resistant. Elhassan (2002) found that there were clear differences among the three Sudanese sheep breeds in their susceptibility to infection with *H. contortus*. Hamari lambs being the least resistant than, Watish and Garag breeds.

1.6.4 Control by grazing management:

The main methods of parasite control through exploiting pasture management have been defined as: preventative, evasive, and diluting (Michel, 1985). Preventative strategies involved putting worm-free animals on clean pasture and suppressing egg output by early treatment in the grazing season. Evasive strategies involved moving calves to a new pasture before the eggs in the current pasture could develop into infective larvae (usually 4 to 6 days). Diluting strategies involve addition of animals that will decrease the parasitic load in the pasture. This can also be accomplished by co-grazing or alternate grazing of different species (Michel, 1985).

In the temperate regions of the world considerable benefits have been achieved in the control of parasites for both sheep and cattle by interchanged grazing between these two species of livestock. These grazing management strategies exploit host specificity, where by the species of parasite that are pathogenic in one host species either do not infect the alternative host, or are less pathogenic. Typical procedures involve alternation of the separate host species at intervals from 2 to 6 months (Barger and Southcott, 1978; Donald *et al*., 1987),

1.6.5 Targeted drenching:

A general feature of parasitic infections in animal flocks or herds is that the distribution of parasites is not even (Roberts and Swan, 1982; Wilson and Grenfell, 1997). That is, a few individuals in flock or herd are infected with the majority of parasites and many animals have few or no parasites (Shaw and Dobson, 1995). Targeted drenching is based on selective treatment of those individual animals that are diagnosed as heavily infected, and with clinical symptoms of the disease. The

FAMACHA system was founded on this concept, and developed in South Africa for the treatment of small ruminants infected with *H. contortus* (Malan and Van Wyk, 1992). The system utilizes clinical identification of anaemia in animals infected with this parasite by the use of a chart indicating the degree of anaemia. Thus, instead of treating the whole flock, only those animals with infections inducing anaemia are treated with an effective anthelmintic (Van Wyk *et al*., 1998; Van Wyk and Bath, 2002; Vatta *et al.*, 2002). This system is only suitable in situations where anaemia is attributable to infection by *H. contortus*. Therefore, conditions causing anaemia other than Haemonchosis could be eliminated for the correct use of the FAMACHA system (Van Wyk and Bath, 2002).

1.6.6 Biological control:

In contrast to other methods of control of nematode parasites of livestock, biological control is directed at the free-living stages, rather than parasitic stages within the host. Many micro-organisms have been identified as predators, pathogens or parasites of nematodes (Waller and Faedo. 1996) and the most promising candidates as possible bio-control agents of animal parasitic nematodes are the nematophagous fungi (Gronvold *et al*., 1993; Waller, 1993). Current research has focused almost exclusively on *Duddingtonia flagrans*, this microfungus has been shown to occur in the same environment that favors larval development and transmission (Thamsborg *et al*., 1999; Larsen, 1999, 2000). Administration of the fungus *Duddingtonia flagrans* as spores (chlamydospores) to sheep or cattle, has shown reduction in a number of larvae of nematodes in feces (Larsen, 2000; Paraud and Chartier, 2003; Paraud *et al*., 2004).

Biological control has many obvious attractions and advantages over other non-chemotherapeutic means of parasitic control. For example, it will

be applicable to the range of nematode parasites not only within, but also between, species of livestock. It will provide the opportunity for livestock producers to capitalize on the increasing demands by consumers for chemical-free livestock products. Finally, because the mechanisms by which fungi kill larvae are complex, it is difficult to envisage developing resistance mechanisms of worms **(**Githigia *et al.*, 1997).

1.6.7 Copper oxide wire particles (COWP):

Studies conducted in sheep in New Zealand (Bang *et al*., 1990) and Australia (Knox, 2002), and in goats in France (Chartier *et al*., 2000), COWP administered as capsules, showed to be effective in reducing the establishment and fecundity of *H. contortus*. The mechanism of action is assumed to be based on the lethal effects on the parasite due to ionic copper liberated from the COWP by the acid secreting mucosa of the abomasum. However, the concentration necessary for an anthelmintic effect and the potential for toxicity in copper for animals exposed to copper accumulating plants, are yet to be established.

1.7 Plants with general anthelmintics:

Medicinal plants have served through ages as a constant source of medicaments for the exposures of a variety of diseases. The history of herbal medicine is almost as old as human civilization. The plants are known to provide a rich source of botanical anthelmintics, anti-bacterial and insecticides (Satyavati *et al*., 1976; Lewis and Elvin-Lewis, 1977). A number of medicinal plants have been used to treat parasitic infections in man and animals (Nadkarni, 1954; Chopra *et al*., 1956; Said, 1969; Akhtar *et al*., 2000).

Although many plants or plant extracts have been reported for their general antiparasitic properties, in this review we will focus on those with specific anthelmintic properties.

In Africa, traditional medicine still represents a major mode of control of parasitism in order to improve both resistance and the resilience of the host. Ibrahim *et al*. (1984) in Nigeria screened 18 plants traditionally used for the treatment of animal and human helminthesis and for anthelmintic activity. Alawa *et al*. (2003) showed that the Nigerian medicinal plant *Annona sengalensis* inhibited *in vitro* significantly the hatching of eggs from *H. contortus*. Also Ademola *et al*. (2007) reported that the Nigerian medicinal plant *Spigelia anthelmia* extract could be applied for the control of helminth in livestock, by the ethnoveterinary medical approach. In Kenya, Kokwaro (1993) listed 21 plants used against hook worms (*Ancylostoma* spp.), six against round worms (without specifying), 22 plants against tape worms (cestodes) and one plant used against strongyloids spp. However, Parker and Palmer (1991) studied the anthelmintic effect of *Alliandra calothyrsus* on *Haemonchus contortus, Trichostrongylus* spp., and *Strongyloides papillosus* infections in sheep. They found no evidence that this forage reduced the population of nematodes present or affected the viability of their eggs. Githiori *et al*. (2002) showed that, the tested extracts of the *Myrsine ofricana* and *Rapanea melanophloeos* were not efficacious against *H. contortus* in sheep. The same authors Githiori *et al*. (2004) evaluated the anthelminthic efficacy of 7 plants used as dewormers by farmers and pastoralists in Kenya. they reported that, no significant differences were observed in mean total worm count or in the number of eggs per female worm between treated animals and the control, also no significant improvement in weight gain was observed in treated lambs. Caroline *et al*. (2005) showed that, the

ground leaves of *Jasminum abyssinicum* were efficacious with significant faecal egg count reduction of 69% at day 7 post treatment. In Zaire, Kasonia *et al*. (1991) identified 32 plants used in traditional veterinary medicine. In Tanzania, Minija (1994) reported that, a herbalist is usually consulted first and then veterinary help is only sought if the resulting treatment is unsuccessful. He collected 103plant species which are used for their curative properties in ethnoveterinary medicine, 23 of these are anthelmintics. In an earlier study, the same author (Minija, 1989) collected 65 plants used in traditional veterinary medicine and of these 7 were classed as anthelmintics. Three of these, *Cissampeles mucromate, Senecio lyratipartitus* and *Croton macrostachys* were classed as very potent anthelmintics by traditional herbalists. In Western Africa, Hounzangbe *et al*. (2005) in Benin screened *in vitro*, the alcoholic extracts of four tropical plants (*Zanthoxylum zanthoxyloides, Newbouldia laevis, Morinda lucida* and *Carica papaya*) and the extract of fagara (*Z. zanthoxyloides*) was found to be less active against *Haemonchus* infective larvae than the extracts of the other plants, however, *N. laevis* was found to be highly and rapidly effective against adult worms. In Uganda, Gradé *et al*. (2008) indicated that *Albizia anthelmintica* is used as part of an integrated management plan for the control of helminths in developing countries. Recently in Ethiopia, Equale *et al*. (2007a) reported that the hydroalcoholic extract of *Coriandrum sativum* showed better *in vitro* activity against adult *H. contortus* than the aqueous one. Both types of the extracts of *C. sativum* inhibited hatching of eggs completely at a concentration less than 0.5 mg ml^{-1}. For *in vivo,* treatment with both doses (0.45 and 0.9 gm kg^{-1}) of *C. sativum* did not help the animal to improve or maintain their PCV, while those treated with albendazole showed significant increase in PCV ($P<0.05$). Significant total worm count reduction was detected only for

higher dose of *C. sativum* compared to the untreated group, significant faecal egg count reduction was not detected for both doses of *C. sativum*. Complete inhibition (100%) of larval development and egg hatching at the maximum concentration tested (50 mg/ml) was obtained only for hydro-alcoholic extract of the fruits of *Maesa lanceolata* and the lowest inhibition (50.33%) was recorded for the hydro-alcoholic extract of the leaves of the same plant. These findings have shown that *M. lanceolata* contains possible anthelmintic compounds (Tadesse, *et al*., 2009). In France, Okpekon *et al*. (2004) identified and collected 17 plants for their evaluation *in vitro* as antiparasitic drugs. They found 10 species to have anthelmintic activities. Marie-Magdeleine *et al*. (2009) for *in vitro* study suggested anthelmintic properties of *Cucurbita moschata* seed against *H. contortus.* In Switzerland, plant extracts identified from ethnoveterinary sources for their anthelmintic properties, were tested in experimentally infected sheep for their activity against gastrointestinal nematodes by Hordegan *et al*. (2003). A 100% reduction was observed in faecal egg count and 72 and 88% reduction of adult *H. contortus* and *Trichostrongylus colubriformis* was observed in sheep offered an ethanol extract of *Fumaria parviflora*. Hordegan *et al*. (2006) reported that, Bromelain, the enzyme complex of the stem of *Ananas comosus* (*Bromeliaceae*), the ethanolic extracts of the seeds of *Azadirchta indica*, *Caesalpinia crista* and *Vernonia anthelmintica*, and the ethanolic extracts of the whole plant of *Fumaria parviflora* and of the fruit of *Embelia ribes* showed anthelmintic efficacy of up to 93% relative to the pyrantel tartrate.

Studies on anthelmintic properties of some medicinal plants were carried out in Brazil by Assis *et al*. (2003),Maciel *et al*. (2006), Costa *et al*. (2006) and Oliveira *et al*. (2009). Assis *et al*. (2003) evaluated *in vitro* five concentrations (3.1, 6.2, 12.5, 25 and 50 mg ml^{-1}) of *Spigelia anthelmia*

extracts. At 50 mg ml^{-1} the ethyl acetate extract inhibited 100% of the egg hatching and 81.2% of the larval development. In a similar way the methanolic extract inhibited 97.4% of the egg hatching and 84.4% of the larval development. Maciel *et al*. (2006) reported that, the seed ethanol extract of *Melia azadarch* was the most active on eggs (LC_{50}= 0.36 mg ml^{-1}) and the leaf ethanol extract showed the best inhibition of larval development (LC_{50}= 9.18 mg ml^{-1}). However, Costa *et al*. (2006) studied the anthelmintic activity of *Azadirrachta indica* on sheep infected with gastrointestinal nematode. Their results showed that, none of the faecal egg count, worm burden, haematocrit values and live weight gain of the treated groups was statistically deferred when compared with the control group. In India and Pakistan many workers investigated the anthelmintic activity of the medicinal plants (Neogi *et al*., 1964; Lal *et al*., 1976; Akhtar and Riffat, 1987; Singh *et al*., 1997; Khunkitti *et al*., 2000; Asha *et al*., 2001; Kahiya *et al*., 2003; Ahmed *et al*., 2004; Jabar *et al.,* 2006). The anthelmintic property of the aqueous extract of the seeds of *Carica papaya* against *Ascaris lumbricoides* and *Ascarida galli* has been evaluated by Dhar *et al*. (1965). The root bark of *Alangium larmarckii* has exhibited good efficacy against the hookworms of dogs and poultry ascarids (Dubey and Gupta, 1968). Sharma *et al*. (1971) reported significant *in vitro* effect of the extracts of *Cucurbita pepo*, *Calotropis gigantea*, *Juglans regia*, *Momordic charantia, Musa paradisaca* and *Scindapsus officinalis* on the motility of mature *H. contortus* of goat origin. Akhtar and Riffat (1984) studied the effect of *Melia azadarach* against naturally acquired gastrointestinal nematodes in goats. The nematodes infecting animals were *Haemonchus, Trichostrongylus, Trichuris* and *chabertia* species. The reductions after dosing the goats with 30 mg ml^{-1} of the powdered fruits were 79, 96 and 99% at 3, 10 and 15 days after treatment respectively. The same authors

(Akhtar and Riffat, 1985) had also studied the effect of the powdered fruit of *Melia azadarach* and its extracts in experimentally infected chickens. The powdered fruit, given at 20 mg kg^{-1} produced a 58% reduction in faecal egg counts 15 days after treatment, and the ethanol extract produced a 68% reduction. Various extracts of *Vernonia anthelmintica* have been tested for their anthelmintic activity. Alcoholic extract has been found to possess maximum anthelminitic activity, followed by etheral extract, whereas, aqueous extract has no anthelmintic activity (Singh *et al*., 1985). The water and ethanol extracts of the whole plant powder of *Fumaria parviflora* at 2 g kg^{1} and morantel tartarate at 0.01 g kg^{-1} were compared for their efficacy against *Trichostrongylus*, *Haemonchus* and *Trichuris* nematode in sheep. The respective reductions in EPG were 99.6±0.13, 29±4, 99.8±0.08 and 99.8±0.3% (Akhtar and Javed, 1985). The water and ethanol extracts of *Nigella sativa* seeds, the powder at 2.5 g kg^{-1} and Niclosamide at 0.1 g kg^{-1} caused 74±4, 99±0.02,99±0.03 and 100±0.6% reduction in EPG of *Moneizia* in sheep (Akhtar and Javed, 1991). The active principles of *N. sativa* have also been evaluated for their anticestodal efficacy in goats. Glycosides (200 mg kg^{-1}), saponinin (200 mg kg^{-1}), Anthraquinones (200 mg/kg) of *N. sativa* and Nilzan at 5 ml 15 kg^{-1} reduced EPG by 94±5, 8±4, 6±3 and 97±4% respectively (Akhtar and Aslam, 1997).The anthelmintic activity of essential oil of *Piper betle* against tapeworms has been found to be superior to that of piperazin phosphate (Garag and Jain, 1992). Satrija *et al*. (1994) showed that, treatment with *Papaya latex* at a dose level of 2, 4 or 8 gm kg^{-1} reduced worm burdens of pigs naturally infected with *Ascaris suum* by 39.5, 80 and 100% respectively compared with those of the non treated controls. Kailani *et al*. (1995) evaluated antifasciolic efficacy of powdered *Nigella sativa* seed, *Fumaria parviflora* aerial part, and *Coesalpinia crista* seeds in

buffaloes. Maximum antifasciolic efficacy, judged on the basis of percentage reduction in EPG was shown by *F. parviflora* at 60 mg kg^{-1} (93.2±0.5%) followed by *C. crista* at 60 mg kg^{-1} and *N. sativa* at 25 mg kg^{-1} (88.2±0.4%) at day 15 post treatment. Tandon *et al*. (1997) studied the *in vitro* activity of root of *Flemingia vestita* against helminth parasites (*Ascaris suum*, *Ascaris lumbricoides, Ascardia galli, Heterakis gallinarum, Raillietina echinobothrida* and *Paramphistomum* spp). Treatment of the parasites with crude extract 50 mg ml^{-1} revealed complete immobilization of the trematode and cestode about 43 and 20 min, respectively. However, the nematode did not show any change in the activity. The effect of Indian mulberry on cellular immune response was shown in sheep drenched daily at the dose level of 0.4 g kg^{-1} or given a single dose of 6 g kg^{-1} of Indian mulberry powder. This treatment increased significantly the total leukocyte and blood eosinophil counts and stimulated proliferation of mast cell in gut (Satrija *et al*., 2001). Methanol extracts of some commonly used plant materials of ethno-veterinary importance in Pakistan were screened for their *in vitro* anthelmintic activity by Igbal *et al*. (2001). Their result revealed that *Zingiber officinale* killed all the test worms (*Haemonchus contortus*) within two hours post exposure thus being 100% effective. *Allium sativum* and *Cucurbita mexicance* were 100% and 83.4% effective respectively. *Ficus religiosa* was 100% effective after 4 h post exposure. Lateef *et al*. (2003) studied *in vitro* and *in viv*o anthelmintic effect of crude aqueous (CAE) and methanol extract (CME) of *Adhatoda vesica* on *H. contortus*. The maximum reduction in EPG (37.4%) was recorded in sheep treated with (CAE) at 3g kg^{-1} body weight on day 10 post treatment (PT) followed by crude powder at 2 g kg^{-1} (33.05%) and CME at 3 g kg^{-1} (25.6%) on day 14 PT. Igbal *et al*. (2005) studied *in vitro* and *in vivo* anthelmintic activity of *Calotropis procera* against *H. contortus*. *In vitro*

studies revealed anthelminthic effect of crude aqueous (CAE) and crude methanolic extract (CME) of *C. procera* on *H. contortus*. For *in vivo* studies, egg count percentage reduction was recorded as 88.4 and 77.8% in sheep treated with CAE and crude powder at 3g kg^{-1} body weight on day 7 and 10 post treatment (PT) respectively. CME was least effective resulting in 20.9% reduction in egg count reduction (ECR) on day 7 PT. Seeds of *Butea monosperma* administered as crude powder at a dose 1, 2 and 3 g kg^{-1} to sheep naturally infected with mixed species of gastrointestinal nematodes showed reduction of 87.4% in eggs per gram of faeces on day 10 after treatment with 3g kg^{-1} (Igbal, 2006 a). Shaik and colleagues (2006) observed an 80% reduction in feacal egg count in goats fed on *Sericae lespedeza* compared with *cynodon dactyon* hay. The *S. lespedeza* fed to goats also had less development of *H. Contortus larvae* in faecal culture, and approximately 70% fewer adult *H. contortus* in their abomasi. The anthelmintic activity of *Zingiber officinale*, commonly known in Pakistan as ginger was described by Igbal (2006 b). Both crude powders and aqueous extracts exhibited anthelmintic effect with respective maximum reduction of 25.6 and 66.6% in egg per gram of faeces on day 10 post treatment. Jabbar *et al.* (2007) reported that, for *in vivo*, the maximum reduction in EPG of faeces was recorded as 93.9 and 82.2% with *Caesalpinca crista* and *Chenopodin album* aqueous methanolic extract at 3.0 g kg^{-1} on day 13 and 5 post treatment respectively. However, levamisole 7.5 mg kg^{-1} showed 95.1-95.6% reduction in EPG. In Saudi Arabia AlQarawi *et al*. (2001) studied the anthelmintic activity of *Calotropis procera* latex in sheep infected with 12000 infective *H. contortus* larvae, and treated with single oral doses of 0.01 and 0.02 mg kg^{-1} body weight of *C. procera*. Egg production was significantly reduced, fewer adult worms were found in the abomasa, the haemoglobin

concentration and serum copper, iron and zinc level were still reduced after therapy with *C. procera.*

Investigations on the anthelmintic activities of the Sudanese medicinal plants were carried out by several workers. Galal *et al*. (1991a) investigated the activity of *Albizia anthelmentica* against experimental infection of *Hymenolips diminuta* in Albino rats. Ibrahim (1992) elucidated the anthelmintic activity of aqueous extracts (0.25 – 50 mg mg^{-1}) from 14 plants which were widely distributed in the Sudan by using the free living nematode *Caenorhabditis elengans.* Khalid (1992) and Elsheikh (1994) showed that, the bark of *Albizia anthelmintica* has a potent activity against snail and different stags of schistosoma. El-Taieb (1995) concluded that the *Cucurbita maxima* seeds are not an efficient antisestodal plant product in chicks infected with *Railletina tetragona*. Osman (2001) studied the efficacy of four medicinal plants (*Piper abyssinica, Jatropha curcas, Ximenia Americana* and *Lonchocarpus laxiflorus*) in chicken experimentally infected with the tape worm *Raillietina tetragona*. The results indicated that, the powder seed of *Piper abyssinica* produced 89.1% efficacy rate at a dose of 4 g kg^{-1}. The ethanolic extract of *P. abyssinica* showed 86.86% efficacy at 500 mg kg^{-1} body weight. The powder seed and ethanolic extract of *Jatropha curcas* showed 89.1 and 62.5% efficacy at a dose of 500 mg kg^{-1} and 100 mg kg^{-1} respectively. The water extracts of *Ximenia americna* and *Lonchocarpus laxiflorus* showed 20 and 15% efficacy at a dose level of 15 g kg^{-1} body weight. Koko *et al*. (2000) evaluated the therapeutic efficacy of *albizia anthelmintica* bark water extract at a dose of 9 g kg^{-1} body weight orally compared with 20 $mgkg^{-1}$body weight of albendazole; the efficacy was found to be 95.5 and 97.7% respectively. The same author studied eight of Sudanese medicinal plants for their molluscicidal activity against *Lymanae natalensis*. Two of them

Albizia anthelmintica bark, water extract, and *Ximenia americana* leaves, water extracts, were the most potent and revealed activity at concentration of 100 ppm (koko, 2005). Adam (2006) evaluated the anthelmintic activity of *Piper Abyssinia* and *Jatropha curcas* against experimental *H. contortus* infection in desert goats. The powder seed of *P. Abyssinia* and *J. curcua* 1 and 0.5 g kg^{-1} body weight produced 57.4 and 43.3% efficacy rates respectively. The powder seed of *J. curcas* and *P. abyssinica* showed a significant reduction in worm burden compared to the infected control. The efficacy of methanol extract of *Randia nilotica* plant in the treatment of mansonial schistosomiasis in comparison with praziquantel drug in mice has been evaluated and resulted in 87 and 59% inhibition of worm load respectively (Nour *et al*., 2006).

1.8 Plants used in this study:

Many species of the plants grow abundantly in the Sudan and are used by the local people for the treatment of various diseases (Adam, 1978), but most of them have not been confirmed in experimental animals. The present study was initiated in order to evaluate the anthelmintic activity of *Balanites aegptiaca* and *Artemisia herba alba*.

1.8.1 *Balanites aegyptiaca:*

1.8.1.1 Taxonomy and morphology:

Balanites aegyptiaca is one of the very useful trees in the Sudan. There are about 25 species of the genus *Balanites* (Trease and Evans, 1978). *B. aegypticae* is the only species of the genus Balanites present in the Sudan (Andrweis, 1952). The classification of Balanites recognized by

Takhtajan (1969) is as follows:

Phylum : Anigospermae

Subphylum : Dicotyledons

Grade : Archiclamydeae

Order : Geraniales

Family : Zygophyllaceae

Genus : Balanites

Balanites aegyptiaca is a large savanna tree widely distributed throughout Africa, along the tropical pelt from Tanzania in the east to Ivory Coast in the west, it is also found in the relatively drier regions of Northern Africa from Mauritania to Nigeria and Ghana, to Egypt across Palestine, Saudi Arabia and India. The drier regions of Kenya, Uganda and Zaire, carry scattered open forests of *B. aegyptiaca* (Suliman and Jackson, 1959). In the Sudan the popular medicinal plant *B. aegyptiaca* is predominant and is found throughout the greater part of the Sudan especially Kordofan, Darfur, Kassala and the Blue Nile regions (Broun and Massey, 1929; Abu El Futuh, 1983).

The macro-morphological characteristics of *B. aegyptiaca* have been described by Broun and Massey (1929). The Balanites tree is a savanna tree which attains a height of more than six meters; it has a spherical shaped crown and a tangled mass of long thorny branches. Leaves are grey green in colour. Flowers are yellow green in colour and the spines are simple. The ripe Balanites fruit is a drupe which is yellow in colour 4 ± 2.5 cm, and the weight is about 10-15 gm per unit. It is composed of skinny brittle epicarb; a fleshy mesocarp, a woody endocarp and a kernel (Abu Alfutuh, 1983). The Balanites fruit is known as Lalobe in Arabic; other names include Heglig berries of Sudan, desert dates and Egyptian myro-balan (Suliman and Jackson, 1959).

1.8.1.2 Medicinal and other uses of *Balanites* fruit:

Various parts of the plant are used locally to exhibit economical importance and medicinal folkloric uses. The folkloric and medicinal application in Africa of *B. aegyptiaca* fruits were mentioned by Watt and Breyer-Brandwijk (1962)) as to be used for washing clothes and hair as lice killer. In eastern Africa it has been used as an insect antifeedent, antimicrobial and molluscicide. The oil from the kernel of the fruit has been used by villagers of Sudan to treat skin diseases. The Balanites fruit is considered as an article of diet among the Zandy and Nilotic tribes of the Sudan, the fruit is also sucked by school children as an article of confectionary. In Uganda, the fruit of the tree is employed as a remedy for sleeping sickness (Cushny, 1908). In Tanganyika, the bark of the tree is used in the treatment of syphilis and roundworm infections and as a fish poison (Bally, 1937). The stem bark has been utilized in India as a purgative, anthelmintic and as a cure for syphilis (Uphoff, 1968). It has been suggested by Watt and Breye-Brandwijk (1962) that the fruit of *B. aegypliaca* is used as a remedy for liver and spleen conditions in certain African countries. The fruit kernel bark, root and branches of *B. aegyptiaca* have proved lethal to mollusca, miracidia and cercariae of *Schistosoma mansoni* (Archibold, 1933; Bashir *et al*., 1984a). The water extract and saponin fraction of *B. aegyptiaca* kernel cake were found to possess a potent mosquitoe larvicidal activity (Zaroug *et al*., 1988). Bashir *et al*. (1984b) found that the saponin of kernel cake of *B. aegyptiaca* has a high antibacterial activity against gram positive bacteria particularly *Bacillus subtilis* and *Staphylococcus aureus*. Mohamed *et al*. (1999) showed that there was a significant effectiveness of the aqueous extract of the bark of *B. aegyptiaca* in increasing serum bilirubin level in experimental obstructive jaundice in rats, thus it has the potential for the treatment of obstructive

jaundice in humans. Koko *et al*. (2000) evaluated the efficacy of *B. aegyptiaca* fruit mesocarp water extract at a dose of 9g kg^{-1} body weight orally in sheep experimentally infected with *Fasciola gigantica* compared with albendazole 20 mg/kg. The efficacy was 93.2 and 97.2% respectively. Also Koko *et al*. (2005) concluded that *B. aegyptiaca* fruit mesocarp could be considered as a highly effective antischistosomal remedy. Khalid *et al*. (2005) reported that the extracts of *B. aegyptiaca* had a moderate biological activity on major promostigates of leishmania.

1.8.1.3 Chemical composition of *Balanites* fruit:

The chemical composition of the fruit of *B. aegyptiaca* has been descried by Bashir *et al*. (1984) and Abu El Futuh (1983). These authors have suggested that, the mesocarp and kernel are the most important parts of the fruit, and the mesocarp is composed of 64-72% carbohydrate, 3.2-6.6% crude proteins, 0.9-1.8% crude fiber and 0.01-0.3% vitamin C. in addition to steroidal saponins. Varshney *et al*. (1977) and Varshney and Jain (1979) isolated five saponins from the pulp and they named them as Balanitesin A, B, C, D and E. The total saponin content was found to be 7.2% in the mesocarp. The fruit, leaf, bark and root of the plant contain saponins, yamogenin, diosgenin and steroidal saponins (Oliver-Bever, 1986).

1.8.2 *Artemisia herba alba*:

1.8.2.1 Morphology and taxonomy:

Kingdom: Plantae – plants

Class : Dicotyledons

Subclass: Asteridae

Order : Asteraces

Family : Asteraceae

Genus : Artemisia

There are 68 species in Artemisia (Bedevian, 1936). The common names of *A. herba alba* is worm wood (English), armoaise (France) and shieh (Arabic). The plant grows in most areas of North Africa.

1.8.2.2 Traditional and medicinal uses of *Artemisia*:

Artemisia herba alba is a well known medicinal plant that has been used in traditional medicine of the Middle East for treating various diseases. It is used as anthelmintic and antidiabetic by the local population. The plant is also used as antimicrobial and poison antidote (Boriky *et al*., 1996). Species of the genus Artemisia are used as carminative, diaphorelics and anthelmintics especially for hook worms in man (Watt and Breyer-Brandwijk, 1962). Jones (1968) reported that the santonin from Artemisia species is well known for its anthelmintic properties against hook worms. Jacob *et al*. (1979) investigated the antibacterial activity of *A. herba alba*. They found that only its essential oil was active against gram positive and gram negative bacteria. Akhtar (1984) evaluated the efficacy of santonin against *Toxocara vitulorum* in buffalo calves which were naturally infected. Santonin is manufactured from *Artemisia maritime* flower head. It has been used for centuries in Pakistan as a broad spectrum anthelmintic. He reported that 15 mg kg^{-1} santonin, 1 g kg^{-1} powdered plant and 88 mg kg^{-1} piperazine have similar effect in reducing the number of eggs per gram of faeces. The anthelmintic activity of various species of Artemisia has been reported against Strongyloides, Nematodirus and Trichostrongylus species (*Artemisia maritime*, Sharma 1993) and *Dipylidium caninum* and Taenia species (*Artemisia maritime*, Narayana *et al*., 1976). The essential oil of *Artemisia pallens* has shown strong anthelmintic activity against *Pheritima posthuma, Taenia solium* and *Ascaris lumbricoides* and was found even

better than piperazine phosphate (Nakhare and Garg, 1991). Also Nagvi *et al.* (1991) reported that the oil from the flowers of *Artemisia scoparia* has exhibited good anthelmintic activity. Igbal *et al*. (2004) found that, *Artemisia bervifolia* whole plant possesses anthelmintic activity against *H. contortus*. Didem *et al*. (2006) suggested that the *Artemisia herba alba* possesses antidiabetic activity similar to that of repaglidin and regular insulin. Also Alshamaony *et al*. (1994) reported that *Artemisia herba alba* showed a significant reduction in blood glucose level, prevent elevation of glycosylatod haemoglobin level and possesses ahypoliposis effect, in addition to the protection against body weight loss of diabetic animals.

1.8.2.3 Chemical composition of *Artemisia*:

Watt and Breyer-Brandwijk (1962) suggested that the medicinal effect of certain artemisiae such as *A. afra* and *A. cina* are due to the presence of santonin, volatile oil and compher. Previous investigation on the non volatile constituents of *A. herba-alba* revealed the presence of sesquiterpene lactones (Macro *et al*., 1994) and flavonoids (Saleh *et al*. (1987) and Salah and Jaeger, 2005) in the aerial parts. Two flavonoids (hispidulin and cirsilineol) from *A. herba alba* were isolated by Sam and Katharina, (2005). Mohmoud *et al*., (2006) reported that, the antifungal activity of *A. herba alba* was found to be associated with two major volatile compounds (carvone and piperitone) isolated from the fresh leaves of the plant.

1.9 Transmission Electron microscopy:

Since the 1960s, transmission electron microscopy (TEM) has been used extensively as a valuable investigative tool in taxonomic and structural studies, including evaluation of drug effects, of both parasitic

flatworms and nematodes (David, 2004). Roy and Tandon (1996) reported severe tegumental alteration and deformities displayed by *Fasciolopsis buski* exposed to20 mg/ml crude extract of *Flemingia vestita*. Beugnet *et al*. (1996) studied the Ultrastructural changes induced *in vitro* by thiabendazole, levamisole, pyrantel and ivermectin in the free living larval stages of two trichostrongyles *(Heligmosomoides polygyrus* and *Haemonchus contortus)*. They found that Thiabendazole induced alteration of the cellular organization especially epithelial cells of the digestive tract. Changes in mitochondria were also seen. Levamisole caused contraction of muscle fibres whereas no specific lesions were observed with pyrantel. Ivermectin caused a hypertrophy of muscular groups. Under electron microscope the *in vitro* treatment of *Raillietina echinobothrida* with the root of *Flemingia vestita*, showed structural alteration in their tegumental architecture, and the tegumental region showed pronounced vacuolization in comparison with the control (Tandon *et al*., 1997). Meaney *et al*. (2004) reported that, there was an increase in the number of autophagic vacuoles, the mitochondria and the granular endoplasmic reticulum remained swollen and lipid droplets were present in the cells of the gut of *Fasciola hebatica* following *in vivo* drug treatment with clorsulon (fasciolicidal).

CHAPTER TWO
MATERIALS AND METHODS

2.1 *In vivo* anthelmintic activity:

2.1.1 Experimental animals:

A total of 28 lambs of sheep of Hamari breed were employed for this study. They were aged 4-5 months at the start of the experiment. All lambs were ear-tagged and housed in clean pens, which were disinfected before admission of the animals. They were fed with Dura and dry groundnut hay. All lambs had a free access to water. Upon their arrival, the animals were subjected to thorough clinical examination and were kept for an adaptation period of one month. During this period they were given two doses of antihelmentic, (Vermiprazole 2% Hipra, Spain) at a dose rate of 2.5 ml/10 kg body weight,one dose of antibiotic (Oxytetracyclin 20%, Avicyclin (Avico) at a dose rate of 1 ml/10kg body weight, one dose of anticoccidial (Sulphadimidine 33% (Injectal, Pharma Swede/Egypt)) and a dose of multivitamins (Univet, Ireland). The animals through examination of faeces during three consecutive weeks before the start of the experiment were proved to be free from internal parasites.The Techniques and recommendation of the World Association for Advancement of Veterinary Parasitology (W.A.A.V.P) second edition of guidelines for evaluating the efficacy of anthelmintics in nematodes of ruminants (Wood *et al*.,1995) were used for this study. Lambs were randomly divided into 7 groups of four lambs each. All groups were

inoculated orally with 3000 third infective larval stages (L_3) of *Haemonchus contortus.*

group 1 was dosed with *Balanites aegyptiaca* one day before infection.

group 2 was infected and then treated with *Balanites aegyptiaca* 25days post

infection (pi).

group 3 was dosed with *Artemisia herba alba* one day before infection.

group 4 was infected and treated with *Artemisia herba alba* 25 days (pi).

group 5 was infected and treated with albendazole 25 days(pi).

group 6was kept as infected nontreated control.

group 7 was kept as uninfected nontreated control.

Samples were collected from all experimental lambs for parasitological, haematological, serological and histopathological examinations.

2.1.2 Parasitological methods:

2.1.2.1 Preparation of pure larval faecal culture of *H. contortus*:

Adult females of *Haemonchus contortus* were obtained from the abomasi of naturally infected sheep encountered during the routine postmortem examination, at Alsalam and Hawari slaughterhouses. The abomasi were opened along their greater curvature, the contents were emptied into a large container. Then the contents were washed several times with tap water through 0.1mm sieve mesh (Analysen sieb –Retsch, Din 188, 5657, Hann. W. Germany). The contents that remained after sieving were transferred into another container and were diluted with tap water. The female worms were individually picked up from the abomasal contents and collected into

petri dishes containing normal saline. The eggs were released from the gravid uteri by crushing the collected worms using a pestle and mortar. The resultant homogenate was then mixed with crushed sheep faeces that were sterilized by heating at 140^{o} C for 2hrs (Roberts and O'Sulivan, 1950). The mixture was wrapped in a piece of gauze and then suspended in a closed marmalade gar containing a small amount of water to provide the media with moisture. The cultures were kept at room temperature ranging between 30-35^{o}C for 7 days (Roberts and O'Sulivan, 1950).

2.1.2.2 Recovery and confirmatory identification of the infective larvae from the faecal cultures:

The Standard Bearman technique (Anon, 1977) was used to recover Haemonchus contortus larvae from the faecal cultures as follows:

The gauze containing the cultured faecal sample was placed in a funnel with a short clamped tube attached to its stem. The funnel was filled with water and the apparatus was left for 3 hours during which the larvae actively migrate out of the faecal material and collected by gravity in the stem of the funnel. The fluid from the stem of the funnel was then collected into a large container. Confirmatory identification of the recovered larvae as infective third larval stage (Plate 1) was carried out according to Anon (1977) and Soulsby (1982). Larvae were stored at 4oC until used.

2.1.2.3 Infection of Experimental animals:

Infective doses of third larval stage were prepared by counting the number of larvae in 1ml of culture material using Eppendorf pipette. Actively mobile larvae were counted for infection.

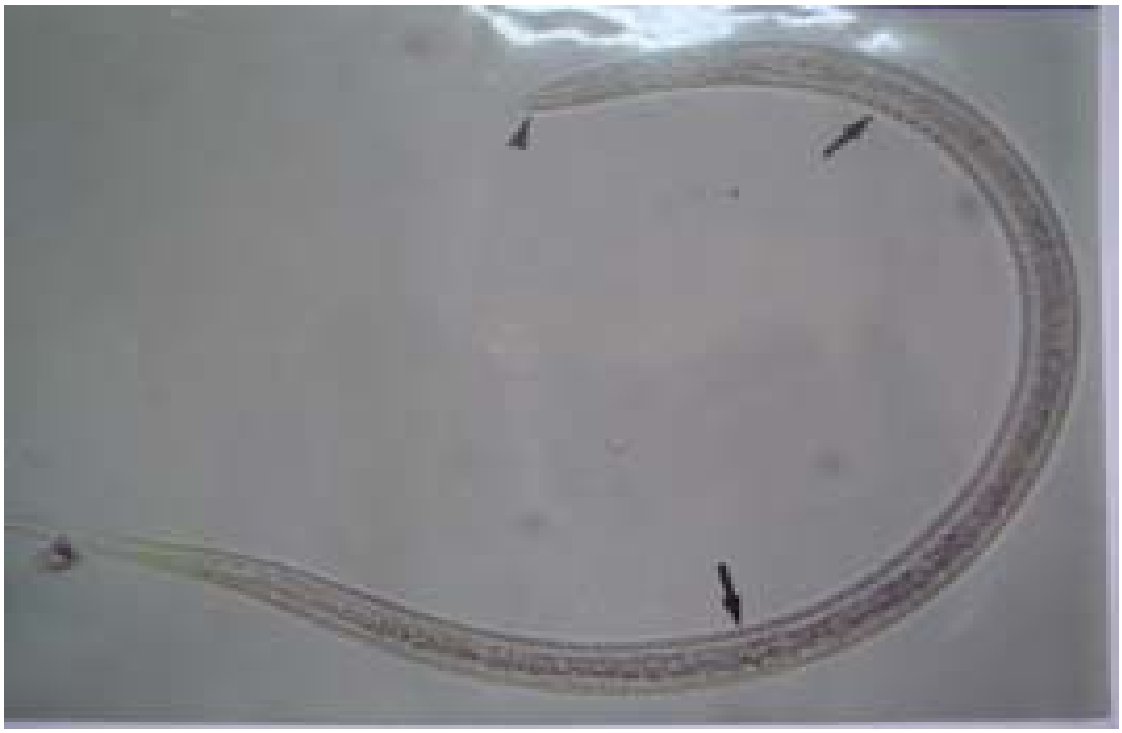


Plate (1): Third larval infective stage (L_3) of *Haemonchus contortus* X100

Showed the corrugated sheath (→) And the tapering anterior end (►).

Each group of experimental animals was dosed by stomach tube and a syringe using the respective dose (3000 L_3/animal) according to the guidelines of the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P) for evaluating the efficacy of anthelmintics in ruminants (Wood *et al*.,1995). After the application of the dose, the tube and syringe were washed with tap water to confirm that all larvae were inoculated.

2.1.2.4 Counting of eggs per gram of fasces:

Fifteen days post infection, fresh faecal samples were collected twice aweek for 5 weeks into plastic bags from the recta of individual lambs. The bags were labeled and taken directly to the laboratory for examination. The number of eggs per gram of faeces (epg) was determined using a modified Mc

Master technique (Thienpont *et al*., 1986).

Two grams of faeces were suspended in 10 ml of water using a pestle and mortar to make up the suspension. To this, 50 ml of saturated sodium chloride solution was added. The suspension was strained through a tea sieve into a flask and then stirred well in order to obtain complete homogeneous distribution of eggs in the liquid. A sample was withdrawn by Pasteur pipette to fill the counting chambers of McMaster slide. The slide was then left for a couple of minutes to allow the eggs to float, and it was then examined under the low power of the microscope.

The eggs in the two chambers were counted and the number of eggs per gram of faeces (epg) was obtained according to the formula:

Epg = <u>number of eggs in the two chambers</u> X 100

Egg count percentage reduction (ECR) was calculated using the following formula:

$$ECR(\%) = \frac{\text{pre-treatment egg count pergram} - \text{post-treatment egg count pergram}}{\text{Pre-treatment egg coun pergram}} X100$$

2.1.2.5 Determination of the adult worm burden of *H. contortus* in the abomasi of experimentally infected lambs:

Infected lambs were sacrificed 7 weeks post infection for determination of the number of adult *H. contortus* in the slaughtered lambs. The abomasi were removed intact and separated from the omasum and duodenum. They were placed in trays and opened along their greater curvature. The abomasal contents were then emptied into a large container. The emptied abomasi were washed thoroughly for several times and the washings were added to the abomasal contents. The contents were then washed several times with tap water through a 0.1mm sieve (Analysen sieb –Retsch, Din 188,5657, Hann. W.Germany). The contents remaing after sieving were transferred into another container and at the end, 10% formalsaline was added to fix the parasites. Recovered worms were then individually picked up, counted and identified. Counts were expressed as number of adult worms per animal. The efficacy of the drug was assessed by the following formula:

$$\text{Efficacy}\% = \frac{\text{average No. of worm in control group} - \text{average No. of worm in treated group}}{\text{Average No. of worm in control group}} X100$$

2.1.2.6 Recovery and counting of larval tissue stages from the abomasi:

For recovery of larval tissue stages, artificial digestion of the abomasal tissue was performed according to the procedure of Herlich (1956).

Abomasi were minced to fine pieces using a Moulinex meat grinder (Moulinex, France). One liter of 1% pepsin solution (10gs of pepsin, EC 3.4.23.1, Sigma, USA, dissolved in one liter of physiological saline) was added to the minced tissues in a 2 liter beaker and stirred with a magnetic stirrer. After homogenization of minced tissues in the pepsin solution, 30 ml of concentrated hydrochloric acid were added to the mixture. The mixture was then left to stir for 2-3 hours at 37^{o}C for digestion of the tissues to be completed. The digested solution was then poured through a 50 μm mesh sieve and washed with tap water to pass most of the digested tissue leaving larval tissue stages and some tissue residues on the mesh, and then preserved using 4% formalin. The preserved volume was washed with tap water using the 50 μm mesh and the whole volume of digested tissue was examined in small aliquots under the dissecting microscope using a marked bottomed petri dish. The removed larval stages were identified under a binocular microscope. The number of counted larvae was expressed as a number of larval tissue stages per abomasum.

2.1.3 Haematological methods:

Blood samples, for the whole blood, were withdrawn from the jugular veins of each lamb twice a week into heparinized vacutainer tubes (Becton Dichinson, France) for 2 weeks before infection and 7 weeks post infection. The blood was used for determination of the following parameters:

2.1.3.1 Determination of haemoglobin (Hb) concentration:

The concentration of Hb was measured by the cyanomethaemoglobin technique using a colorimeter (Ciba corning, England). The method depends on the conversion of Hb by Drabkein's solution (o.2 gm potassium cyanide, 0.05gm of potassium ferric cyanide and one gram of sodium bicarbonate per liter of distilled water) to acyanomethemoglobin. The Hb concentration was measured in gms/L according to the formula:

$$\text{Hb g/L} == \frac{\text{Test}}{\text{Stander}} \times \text{concentration of stander}$$

2.1.3.2 Packed cell volume (PCV):

Blood samples were drawn into capillary tubes then sealed at one end with cristaseal, and centrifuged at 3000 r.p.m. for 5 minutes using a microhaematocrit centrifuge (Hawksley and Sons ltd, England). The PCV was read in a microhaematocrit reader (Hawksley and Sons ltd, England). The reading was expressed as percentage of packed red cells to the total volume of the whole blood.

2.1.3.3 Red blood corpuscles (RBCs) count:

Erythrocytes (RBCs) were counted in an improved Neubauer haemocytometer (Hawksley and Sons ltd, England), using Hayem's solution as a diluent fluid consisting of 0.5g mercuric chloride, 5g sodium sulphate and 1.0g sodium chloride and the whole was made up to 200 ml with distilled water. The number of RBCs/liter blood was determined as described by Jain (1986).

2.1.3.4 White blood cells (WBCs) count:

White blood cells were counted in an improved Neubauer haemocytometer using Turk's solution as a diluent fluid, which consisted of 1.0% glacial acetic acid coloured with Gentian violet. The number of WBCs/litre of blood was counted as described by Jain (1986).

2.1.3.5 Differential WBCs count:

Differential leukocytic count was performed on thin blood films that were made from freshly drawn blood and stained with Leishman's stain. At least 100 leukocytes were counted in every blood smear in Battlement method (Jain, 1986) and the percentage of each cell type was recorded.

2.1.4 Serum biochemical examination:

Blood samples, for serum separation, were collected from the jugular vein into plain vacutainer tubes (Becton_Dickinson , France).The tubes were left to stand for one hour at room temperature and then overnight at $4^{o}C$ to allow ample time for the clot to shrink. Sera were centrifuged at 3000 r.p.m. for 5 minutes, aspirated by Pasteur pipettes and were transferred into Eppendorf tubes. They were labelled and stored at $-20^{o}C$ until used for analysis.

2.1.4.1 Analytical methods:

All serum constituents were measured by using fully automated Roche Diagnostic Hitachi 902 Analyzer apparatus (total protein, albumin, phosphorus and calcium) and Electrolyte analyzer ISE300 (Sodium and

potassium) in the Research and laboratory Unit of Khartoum Teaching Hospital.

2.1.4.1.1 Roche Diagnostic Hitachi 902 Analyzer:

It is an analyzer which is used to report test results on various body fluid samples for a wide range of analysis. It is fully automatic, computerized to perform potentiometric and photometric assays. The apparatus consists of photometric measuring system, analytical processing unit, LCD touch screen and printer. The analyzer is characterized by its ability to perform 200 photometric tests per hour and refrigerated storage for 40 reagent containers.

2.1.4.1.2 Procedure:

For the determination of each parameter, 0.5 ml of the serum was put in Hitachi sample cups. The sample cups were arranged in a specific place and registered in the screen. Thereafter, the machine starts, and the results are printed in ten minutes.

2.1.4.2 Determination of total serum protein:

Total serum protein was determined by Hitachi 902 Analyzer using Buiret method.

Test principle:

Colorimetric assay

•Sample and addition of R1(blank reagent).

•Addition of R2 (biuret reagent)and start of the reaction:

Divalent copper reacts in alkaline solution with protein peptide bonds to form the characteristic purple-coloured biuret complex. Sodium potassium tartrate prevents the precipitation of copper hydroxide and potassium iodide prevents autoreduction of copper.

$$\text{Protein} + Cu^{2+} \xrightarrow[\text{solution}]{\text{alkaline}} \text{Cu-prtein complex}$$

The colour intensity is directly proportional to the protein concentration which can be determined photometrically.

Reagent - working solutions:

R1: Sodium hydroxide:400mmol/L;potassium sodium tartrate:89mmol/L

R2: Sodium hydroxide:400mmol/L;potassium sodium tartrate:89mmol/L; potassium iodide:61 mmol/L; copper sulfate:24.3 mmol/L.

Procedure:

The procedure is mentioned in 2.1.4.1.2.

2.1.4.3 Determination of serum albumin:

The serum albumin concentration was determined by Hitachi 902 Analyzer using Bromocresol green method (BCG).

Test principle:

Colorimetric assay with end point method**.**

•Sample and addition of R1(buffer).

•Addition of R2 (substrate) and start of the reaction:

At aPH value of 4.1 albumin displays a sufficiently cationic character to be able to bind with bromcresol green (BCG), an anonic dyestuf, to form a blue-green comlex.

$$\text{Albumin} + \text{BCG} \xrightarrow{\text{pH 4.1}} \text{albumin BCG complex}$$

The coulor intensity of the blue green colour is directly proportional to the albumin concentration and can be determined photometrically.

Reagent - working solutions:

R1: citrate buffer**:** 95 mmol/L, pH 4.1; preservative

R2: citrate buffer**:** 95 mmol/L, pH 4.1; bromocresol green:0.66 mmol/L; preservative

Procedure:

The procedure is mentioned in 2.1.4.1.2.

2.1.4.3 Determination of Serum globulin:

The serum globulins (gm /dL) were determined by the deduction of the values of serum albumin from the total serum protein values.

2.1.4.4 Determination of Serum Phosphorus:

Phosporus was measured by Hitachi 902 Analyzer according to phosphomolybdate/ UV reaction.

Test principle:

End point method with sample blanking

•Sample and addition of R1(reagent blank).

•Addition of R2 (phosphate reagent) and start of the reaction:

Inorganic phosphate forms an ammonium phosphomolybdate complex having the formula $(NH4)_3[PO_4(MoO_3)_{12}]$ with ammonium molybdate in the presence of sulfuric acid. The complex is determined photometrically in the ultraviolet region (340 nm).

Reagent - working solutions:

R1: Sulfuric acid: 0.36 mol/L; detergent

R2: Ammonium molybdate**:** 3.5 mmol/L; sulfuric acid:0.36 mol/L; sodium chloride: 150 mmol/L.

Procedure:

The procedure is mentioned in 2.1.4.1.2.

2.1.4.6 Determination of Serum calcium:

Calcium determination by Hitachi 902 Analyzer is based on the reaction of calcium with o-kresolphthalein complexon in alkaline solution.

Test principle:

End point method with sample blanking

•Sample and addition of R1(buffer).

•Addition of R2 (chromogen) and start of the reaction:

Calcium+o-kresolphthalein complexon $\xrightarrow{\text{alkaline solution}}$ calcium o-kresolphthalein complex

The colour intensity of the resulting purple solutions is directly propotional to the calcium concentration and is measured photometrically.

Reagent - working solutions:

R1: buffer :1 mol/L, pH 10.6.

R2: o-kresolphthalein complexon: o.3 mmol/L; 8-hydroxyquinoline: 13.8 mmol/L; hydrochloric acid: 122 mmol/L.

Procedure:

The procedure is mentioned in 2.1.4.1.2.

2.1.4.5 Determination of Serum Sodium and Potassium:

Sodium and potassium were measured using electrolyte analyzer ISE300 (Ion Selective Electrode). The results were obtained automatically.

Measuring principles:

The measurement method is called standard comparison. It uses two kinds of standard solutions; one is for the calibration of the base point, and the other is for the calibration of the slope. The result is obtained from the potentials of the sample and two standard solutions.

Following are the equations:

$C_X = C_A * Exp [(E_X-E_A) /S]$

$S = E_B-E_A / log (C_B/C_A)$

C_X, E_X: the concentration and potential of the sample

C_A, E_A: the concentration and potential of standard A

E_B, C_B: the concentration and potential of standard B

S: the slope electrode

2.1.5 Live body weight Gain:

Live weight was recorded for each animal for two weeks before infection. Then the lambs were individually weighed once weekly in the morning during the whole experimental period using Salter / Trade Spring Balance No. 235.T.

2.1.6 Pathological methods:

2.1.6.1 Post mortem examination:

Post mortem examination was performed at the end of the experiment. The abomasi were legated, removed and carefully examined for the presence of worms and related lesions. All mature and immature worms were recovered and counted as described previously. The sections of abomasi were immediately collected and fixed in 10% nutrient formal saline.

2.1.6.2 Histopathological examination:

The formalin fixed specimens were prepared for routine histopathological processing and staining as described by Drury and Wallington (1967). The tissues were first dehydrated with graded

concentration of alcohol, cleared with xylol and embedded in paraffin wax. Microtomy was performed by rotatory microtome (Baired and Totlock, England), and the sections (5 microns thickness) were stained with haematoxylin and eosin (H&E). The stained sections were covered with slips, fixed with Canada balsam and examined under Leitz light microscope.

2.2 Treatment methods:

2.2.1 Preparation of plant extracts:

Selection of these plants *Balanites aegyptiaca* (plate 2) and *Artemisia herba alba* (plate 3) was made on the basis of information gathered about their use in traditional medicine. They were collected from Omdorman market.

2.2.1.1 Preparation of water extraction:

***Artemisia herba alba*:** the dried powder of *Artemisia herba alba* was macerated in distilled water in a ratio of 1:5 w/v in a large conical flask with occasional shaking for the first 4 hours, then allowed to stand overnight at room temperature. The extract was filtered using cotton wool and the filtrate was freeze-dried using alpha 1-12 freeze-Drier. The freeze-dried residue was stored at 4^0C till further use.

Balanites aegyptiaca: The outer epicarp of *Balanites aegyptiaca* fruits was removed and the fleshy mesocarp was scrapped off by means of a sharp knife. Simple maceration technique was applied. The *Balanites aegyptiaca* fruit mesocarps (9g/kg) were macerated in a distilled water in a large conical flask then allowed to stand overnight at 4^0C then they were shacked occasionally before dosing to animals.

Plate 2: *Balanites aegyptiaca* fruits

Plate 3: *Artemisia herba alba* shoot

2.2.1.2 Preparation of methanol extraction:

The extraction was carried out according to the method descried by (Harborne,1984).Three hundred grams of mesocarp of the fruit of *Balanites aegyptiaca* and 500 grams of the powder of *Artemisia herba alba* were extracted with 70% methanol for 12 hrs using a soxhlet extracter apparatus. Extraction was carried out till the colour of the solvent returned colorless. The extracted substance was filtered and put over-night in rotating water evaporator for the evaporation of the solvent to give the methanolic extract. Extracts were left until complete dryness. The extracts were scraped off and stored in an airtight container at 4^0C until used. These extracts were obtained from the laboratory of the plant extraction at the Medicinal and Aromatic Plants Research Institute (MAPRI).

2.2.2 Dosing of the animals:

Animals in Group**s** (1, 2) were treated orally with fruits of *Balanites aegyptiaca* with a dose of 9g/kg body weight dissolved in 250 ml of water in a large bottle and drenched to the animals. Animals in group (3, 4) were treated orally with aqueous extract of *Artemisia herba alba* with a single dose of 3g/kg body weight dissolved in 250 ml of water in a large bottle and drenched to the animals. Group (5) were treated with a single oral dose of 5mg/kg of albendazol (2.5% Ganadexil Invesa Industrial Veterinaria, Barcelona, Espana).

2.3 *In vitro* anthelmintic activity:

For *in vitro* studies, *H. contortus* proved to be a useful worm because of its longer survival in Phosphate Buffered Saline (PBS). The *in vitro* trials for

anthelmintic activity of both plants (*Artemisia herba alba* and *Balanites aegyptiaca*) and albendazole were carried out using the motility assay of the adults and third larval stages.

2.3.1 Adult motility assay:

Adult motility assay was conducted on mature *H. controtus* worm following Sharma *et al*. (1971). Briefly, the mature worms were collected from the abomasi of freshly slaughtered sheep in the Hawari abattoir. Immediately after slaughtering, the abomasi were collected and transported to the laboratory. Adult worms were collected after giving the longitudinal incision along the greater curvature of the abomasi of naturally infected sheep. The worms were picked manually and placed in Petri dishes. Ten actively moving worms were exposed in triplicates to each of the following treatments in separate Petri dishes at room temperature (25 – 30^{o}C).

1. *Balanites eagyptiaca* water extract at 5, 10, 25and 50 mg/ml
2. *Balanites eagyptiaca* methanol extract at 5, 10, 25and 50 mg/ml
3. *Artemisia herba alba* water extract 5, 10, 25 and 50 mg/ml
4. *Artemisia herba alba* methanol extract 5, 10, 25 and 50 mg/ml
5. Albendazole 5 mg/ml
6. PBS (0.9% phosphate buffer saline (8 g NaCl, 0.34 g KH_2PO_3 and 1.21g K_2HPO_4 in 1L of distilled water, pH 7-7.3).

The inhibition of motility and/or mortality of the worms subjected to the above treatments were used as a criteria for anthelmintic activity. The dead worms were easily recognized by their straight flat appearance with no movements at the head and tail regions of the body. The motility was recorded after 0, 2, 3, 4, 6 h intervals.

2.3.2 Larval motility assay:

Adult female parasites of *H. contortus* were collected from the abomasi of infected sheep obtained from Alhawari Abattoir. The worms were washed and crushed to liberate eggs. The eggs were then cultured in sterilized sheep faeces for 8 days at room temperature. At the end of 8th days, infective larvae were harvested using standard Bearman techniques. The larval suspension was diluted in water. Approximately 10 larvae in 0.5 ml of water were placed in each test tube and exposed in triplicates to each of the following treatments in separate test tube at 4^{o}C (Al-Qarawi *et al*., 2001).

1. *Balanites eagyptiaca* water extract at 5, 10, 25, 50 mg/ml
2. *Balanites eagyptiaca* methanol extract at 5, 10, 25, 50 mg/ml
3. *Artemisia herba alba* water extract at 5, 10, 25, 50 mg/ml
4. *Artemisia herba alba* methanol extract at 5, 10, 25, 50 mg/ml
5. Albendazole 5 mg/ml
6. Water as control.

Observations on the motility of the larvae at 2, 6, 12, 24 h were made under light microscope (X10).

2.4 Electron microscopy:

After exposure to the above treatments, dead worms were fixed in 3% gluteraldehyde diluted in 0.15 m phosphate buffered saline (PBS) at pH 7.3 for 24 h at 4^{o}C, followed by post fixation using 1% osmium tetroxide OSO_4 in PBS for 2 h. Samples were dehydrated in increasing concentrations of ethanol (ethanol 70^{o} for 10 min, 95^{o} for 20 min and 100^{o} for 20 min) and progressively embedded in Epon 812 resin, the blocks were cut in fine sections from 90 – 100 nm thick using an ultramicrotom (Leica – Germany) and mounted on

parlodion coated copper grids. These ultrathin sections were stained with 4% uranyl acetate in aqueous solution for 20 minutes followed by 0.4% lead citrate for 10 min; the sections were examined under JEOL-electron microscope.

2.5 Statistical analysis:

Data collected from various experiments were subjected to appropriate general linear model (GLM) procedure of the statistical analysis using the SAS package (SAS, 1998). Analysis of variance (ANOVA) and mean separation were performed using Duncan's New Multiple Range Test and REGWQ. Geometric means of worm and egg count data were calculated after transformation of the data to log 10 (x +1) to normalize variance. Significant level was taken at $P \leq 0.05$.

CHAPTER THREE

RESULTS

3.1 *In vivo* anthelmentic activity:

3.1.1 Clinical observations:

No clinical signs were observed by any of the uninfected control group during the experimental period. On the other hand all infected groups exhibited signs of haemonchosis as they became generally in-appetent, dull and lethargic. No evidence of toxicity of *Balanites aegyptiaca* or *Artemisia herba alba* was recorded on the experimental doses during or after treatment. Also no abnormal behavioral change was observed after treatment of the animals with the different plant preparations. However, animals treated with *B. aegyptiaca* suffered from transient diarrhea on the first day of the treatment. Lambs treated with albendazole showed gradual improvement in their appetite and general condition and they appeared healthy. Also those treated with *A. herba alba* and *B. aegyptiaca* after infection showed improvement in their condition when compared with untreated control group, but less than those treated with albendazole, while lambs treated with *A. herba alba* and *B. aegyptiaca* before infection didn't show any significant clinical manifestation when compared with untreated control.

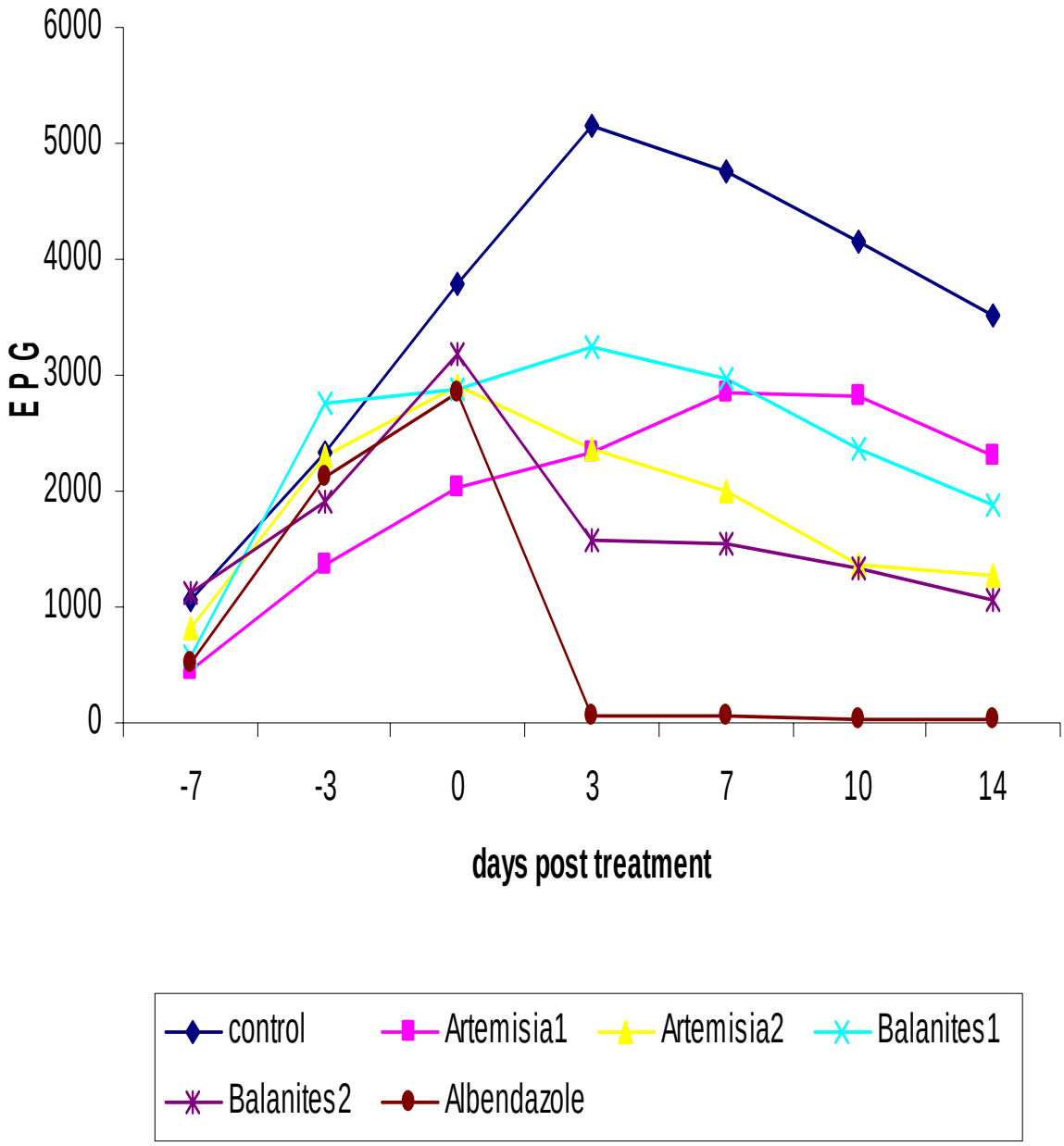
3.1.2 Parasitological findings:

3.1.2.1 Egg count per gram of faeces (epg):

None of the uninfected control lambs voided eggs in their faeces throughout the experimental period. In all infected groups, egg voidance started at day 17 post infection. The mean number of eggs per gram of faeces of infected and treated lambs with water extract of *A. herba alba* and *B. aegyptiaca* fruit mesocarp were shown in Fig. (1).

Animals that were treated with *A. herba alba* and *B. aegyptiaca* before infection, showed slight decrease in epg when compared with the untreated control, but this decrease was not statistically significant. However, All animals treated after infection showed reduction in epg of faeces after treatment especially in albendazole group (standard anthelmentic) when they were compared with the untreated control. A significant decline in EPG was observed from day3 post treatment (pt) until day 14 pt in albendazole and *B.aegyptiaca* and from day 7 to 14 pt in *A. herba alba* when compared with the untreated control.

The Egg Count Reduction percent (ECR %) was shown in table (1). Both *B. aegyptiaca* and *A. herba alba* exhibited significant ($P \leq 0.05$) anthelmentic activity compared with the untreated control. The maximum reduction in faecal egg count was recorded on day 14 post treatment as (69.6%) with *B. aegyptiaca* which was dosed post infection, followed by



1. Treated before infection **2. Treat post infection**

Fig . 1 Mean number of eggs per gram of fasces of experimental lambs

Table (1): Egg count percent reduction (±SE) of lambs after treatment with *Balanites aegyptiaca* and *Artemisia herba alba*

Treatments	Days post treatment			
	Day 3	Day 7	Day 10	Day 14
control	-12.1% c ±12.56	-30.2% c ± 22.2	-20% c ±12.44	5.8% a ±9.23
Balanites aegyptiaca	44.1% b ±7.75	48.3% b ±7.58	51.8% b ±8.38	69.6% b ±4.33
Artemisia herba alba	-11.2% c ±12.3	30.2% b ±12.27	53% b ±4.49	61% b ±6.42
Albendazole	96.1% a ±2.28	96.6% a ±1.96	97.7% a ±1.69	98.5 b ±0.88

* Means (±SE) followed by the same letter in each column are not significantly different at 5% level based on REGWQ.

(61%) ECR with *A. herba alba*. On the other hand, albendazole showed (98.5%) in ECR. No significant difference was shown in ECR within the two plants (*B. aegyptiaca* and *A. herba alba* dosed after infection). However, Albendazole showed significant difference compared with the two plants**.**

3.1.2.2 Total adult *H. contortus* burden:

The arithmetic and geometric means of the total numbers of adult *H. contortus* (Plats 4(a,b),5 and 6) recovered from the abomasi of infected and treated lambs were shown in tables (2and 3). The establishment of infected untreated control group was 25%. All treated lambs showed reduction in total worm burden when compared with the untreated control group.

In animals that were treated with *A. herba alba* and *B. aegyptiaca* before infection, the mean numbers of recovered worms (table2) was lowest in animals treated with *B. aegyptiaca* (437.75 worms.) followed by animals treated with *A. herba alba* (508.25 worms.). However, the untreated control group showed (750.5) worms. On the other hand, lambs that were treated post infection showed lowest worm counts compared with those treated before infection as well as untreated control. The mean numbers of recovered worms (table3) was lowest with albendazole group (19.5 worms.), followed by animals treated with *B. aegyptiaca* (225.67 worms.), and *A. herba alba* (281.25 worms.)

Plate 4: (a) *Haemonchus contortus* anterior end showing the cervical papillae (→) X 100

Plate 4: (b) *Haemonchus contortus* anterior end showing the lancet (→) X 250

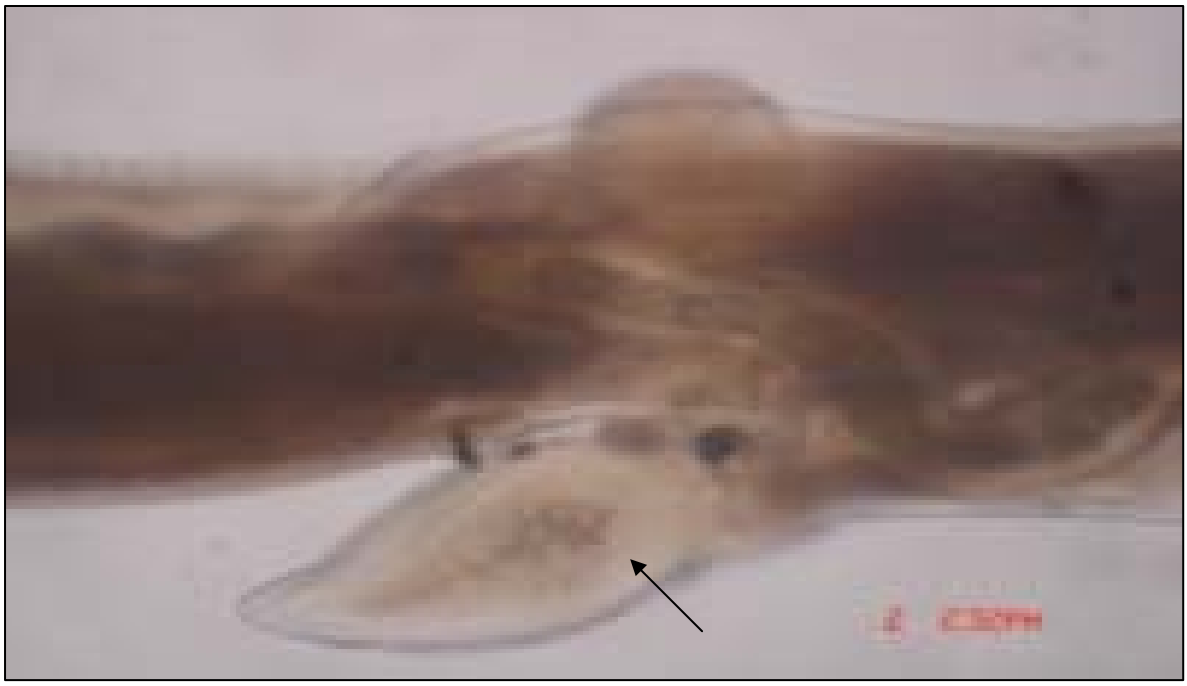


Plate 5: *Haemonchus contortus* (female) showing the vulva flaps (→) X 250

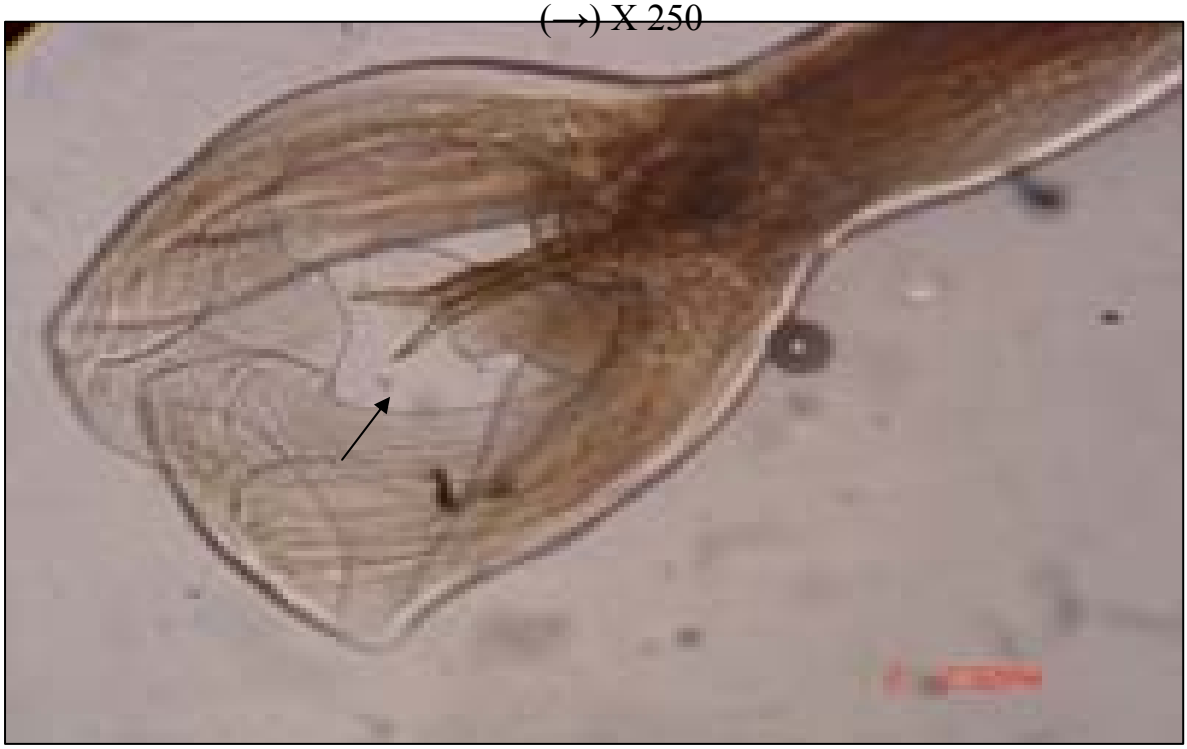


Plate 6: *Haemonchus contortus* (male) showing the Y shaped dorsal ray (→) X 250

Table (2): Mean number (±SE) of adult *Haemonchus contortus* in the abomasi of lambs treated before infection.

treatment	male		female		total	
	Arithmetic	Transformed	Arithmetic	Transformed	Arithmetic	Transformed
control	424 ±45.8 (14.1%)	2.62 ±0.04 a**	326.5 ±38 (10.9%)	2.5 ±0.05 a	750.5±78.8 (25%)*	2.86 ±0.04 a
Artemisia herba alba	262.25±29 (8.74%)	2.41 ±0.04 a	246±27.1 (8.2%)	2.38 ±0.04 a	508.25±40.6 (17%)	2.69 ±0.04 a
Balanites aegyptiac *a*	231.25±43.9 (7.7%)	2.33 ±0.08 a	206.5 ±38 (6.9%)	2.29 ±0.08 a	437.75±75.8 (14.6%)	2.61 ±0.08 a

*Numbers in parenthesis indicate establishment per cent.

** Means (±SE) followed by the same letter in each column are not significantly different at 5% level based on REGWQ.

Table (3): Mean number (±SE) of adult *Haemonchus contortus* in the abomasi of lambs treated post infection.

Treatments	Male		Female		Total	
	Arithmetic	**Transformed**	**Arithmetic**	**Transformed**	**Arithmetic**	**Transformed**
Control	424 ±45.8 **a****	2.62 ±0.04 **a**	326.5 ±38 **a**	2.5 ±0.05 **a**	750.5±78.8 **a**	2.86 ±0.04 **a**
Artemisia herba alba	155±8.17 **b**	2.1 ±0.01 **b**	126.25±24 **b**	2.08 ±0.05 **b**	281.25±32.1 **b** (62.5%)*	2.4 ±0.03 **b**
Balanites aegyptiaca	120 ±7.5 **b**	2.08 ±0.02 **b**	105.67±2.4 **b**	2.02 ±0.009 **b**	225.6±9.93 **b** (69.93%)	2.35 ±0.01 **b**
Albendazole	14.5±3.12 **c**	1.15 ±0.1 **c**	5 ±1.77 **c**	0.72 ±0.12 **c**	19.5±4.69 **c** (97.4%)	1.27 ±0.11 **c**

*Numbers in parenthesis indicate efficacy per cent.

**Means (±SE) followed by the same letter in each column are not significantly different at 5% level based on REGWQ.

Lambs that were treated with *B. aegyptiaca* post infection showed (69.9) % efficacy when compared with the untreated control. While those treated with *A. herba alba* showed (62.5%) efficacy. However, lambs treated with albendazole post infection showed (97.4%) efficacy when compared with the untreated control group.

3.1.2.3 Larval Tissue Stages Count:

No larval tissue stages were recovered from the abomasal digest of all groups of experimental animals.

3.1.3 Haematological findings:

All haematological parameters (PCV, Hb, RBCs, WBCs) recorded from the uninfected control lambs were found to fluctuate within the normal range throughout the experimental period. Observations on haematological parameters in all groups were as follows:

3.1.3.1 Packed Cell Volume (PCV):

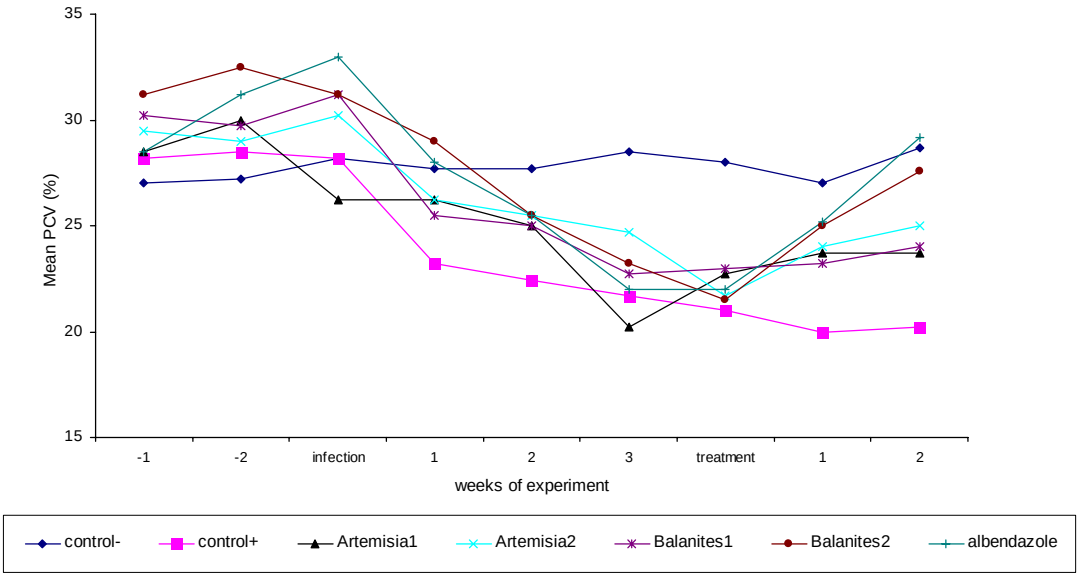
The mean PCV values for experimental animals were shown in Fig. 2. At two weeks before infection there were no significant differences recorded between all the groups. A progressive decline in PCV in all infected lambs was observed from the first week post infection (pi) up to

the forth week post infection but without significant differences between the treated and untreated control group. Slight increase in PCV values was observed in weeks (5, 6) post infection in all treated groups when compared with the untreated control group.

The groups which were treated with *B. aegyptiacea* and *A. herba alba* before infection didn't show any significant difference in PCV values throughout the experimental period when compared with the untreated control group; but in the case of *B. aegyptiaca* there was a significant increase in PCV ($P<0.05$) at week 6 pi. Thus the mean PCV values at week 6 (pi) was (27.2%) in *B. aegyptiaca*, (23.7%) in *A. herba alba* and (20.2%) in untreated control group. On the other hand, animals that were treated with *B. aegyptiaca, A. herba alba* and albendazole post infection, showed significant increase in PCV ($P<0.05$) at week 5 and 6 post infection (days 7, 14 post treatment (pt)). However the mean PCV values on day 14 pt was (27.7%) in *B. aegyptiaca*, (28%) in *A. herba alba* and (29.2%) in albendazole.

3.1.3.2 Haemoglobin concentration (Hb):

The mean Hb concentrations of experimental animals were depicted in Fig. 3. The Hb concentrations in all infected lambs showed a sharp

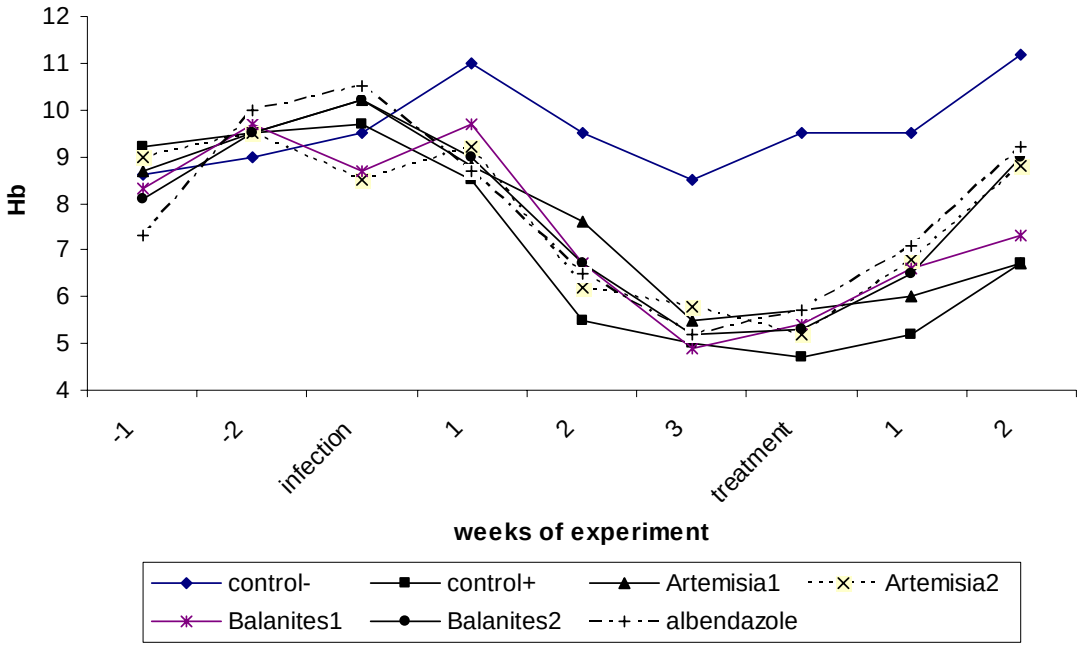


1 treated before infection 2 treated post infection

Fig. 2. Mean PCV values of experimental lambs

decline after the first week post infection(pi) and continued until the forth week pi, after which an increase occurred in animals which were treated after infection. However, in lambs that were treated before infection, very slight increase was observed at week 6pi. No significant differences were observed in Hb values from the two weeks before infection to the first week post infection in all the groups when compared with the uninfected control group. However, a significant reduction in Hb concentrations was shown in all infected groups from the second week pi to the week 6 pi when compared with the uninfected control groups.

Animals which were treated with *B. aegyptiaca* and *A. herba alba* before infection showed slight decrease in Hb concentration at week 6 pi but, this decrease was not significant when compared with the untreated control group. The mean Hb concentrations was (7.3 g/dL) in *B. aegyptiaca*, (7.6 g/dL) in *A. herba alba* and (6.7 g/dL) in untreated control group. Animals that were treated with *B. aegyptiaca, A. herba alba* and albendazole post infection, a significant increase ($P<0.05$) in Hb concentrations occurred at week 6 pi (day 14 pt). The mean Hb concentrations on day 14 pt was (9 g/dL), (8.8 g/dL) and (9.2 g/dL) in *B. aegyptiaca, A. herba alba* and albendazole respectively.



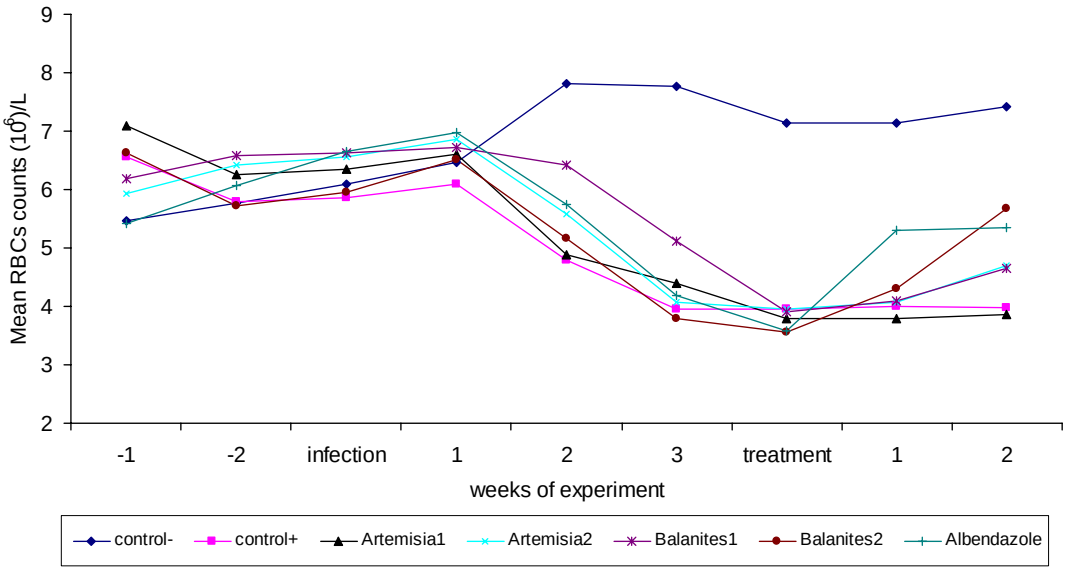
1. Treated before infection **2. Treated post infection**

Fig 3 Mean values of HP Concentrations of experimental lambs

3.1.3.3 Red blood corpuscular counts (RBCs) x 10^6/L:

The mean red blood corpuscular counts of the experimental lambs were shown in Fig. 4. The RBCs count from all infected groups declined at the start of the experiment (week 1, 2 pi) and remained lower than those of the uninfected control, then they started to increase after week 5pi to reach the normal values of the uninfected control group.

In animals which were treated with *A. berba alba* and *B. aegyptiaca* before infection, no significant differences were observed throughout the experimental period when they were compared with untreated control group. However, the *B. aegyptiaca* showed significant differences in RBCs count at week 2 pi. The RBCs count was (4.65 x10^6/L) in *B. aegyptiaca*, (3.86 x10^6/L) *in A. herba alba* and (3.98 x10^6/L) in untreated control group. On the other hand, in lambs that were treated with *A. herba alba*, *B. aegyptiaca* and albendazole post infection, significant differences were observed at week 6 pi (day 14 pt) in *B. aegyptiaca* and at weeks 5, 6 pi in albendazole. However, *A. herba alba* didn't show any significant difference when compared with the untreated control group. The mean RBCs count on day 14 pt was (5.6 x 10^6 /L), (4.7 x 10^6 /L) and (5.36 x 10^6 /L) in *B. aegyptiaca, A. herba alba* and albendazole respectively.



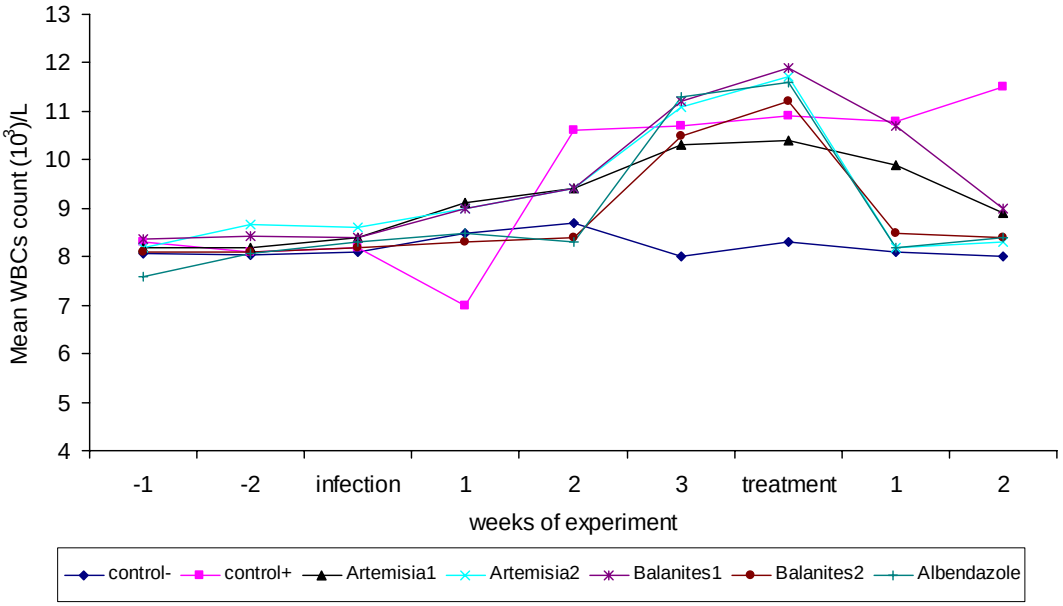
1 treated before infection 2 treated post infection

Fig. 4. Mean numbers of RBCs count per litre of blood of experimental lambs

3.1.3.4 White blood corpuscular counts (WBCs) X 10^3/L:

The weekly mean numbers of WBCs counts of experimental lambs were shown in Fig. 5. The WBCs counts of infected lambs started to increase from the first week pi to reach a peak count at the forth week pi when compared with the uninfected control. A slight decrease was observed at week 5 pi to reach values similar to those of the uninfected animals.

In lambs which were treated with *A. herba alba* and *B. aegyptiaca* before infection, no significant differences were shown in WBCs count throughout the experimental period when compared with the untreated control group, except in week 4 pi in *A. herba alba* and week 6 pi in *B. aegyptiaca* and *A. herba alba* where significant differences occurred. The mean WBCs count at week 6 pi were (9.25 x 10^3/L), (8.75 x 10^3/L) and (8.67 x 10^3/L) in *B. aegyptiaca, A. herba alba* and untreated control groups respectively. Also, in animals that were treated with *A. herba alba, B. aegyptiaca* and albendazole post infection, the significant differences were shown only in weeks (5, 6 pi) when compared with the untreated control group. The mean WBCs counts on day 14 pt were (8.4 x 10^3/L) in *B. aeygptiaca*, (8.32 x 10^3/L) in *A. herba alba* and (8.43 x 10^3/L) in albendazole .



1 treated before infection

2 treated post infection

Fig. 5. Mean numbers of WBCs count of experimental lambs

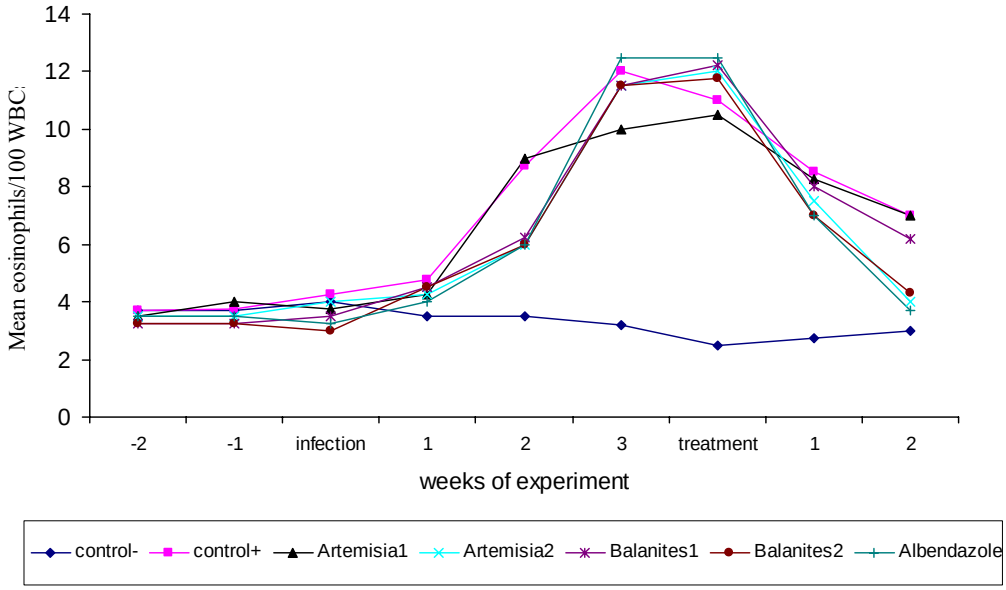
3.1.3.5 Differential WBCs count:

3.1.3.5.1 Eosinophils Count:

The mean percentages of eosinophil counts of experimental lambs were depicted in Fig. 6. The eosinophil counts from all infected groups showed a sharp increase after the second week post infection and then they were decreased thereafter until the end of the experimental period. The significant increase was shown in weeks 3, 4 and 5 post infection when compared with the uninfected control ($P \leq 0.05$).

In animals which were treated with *A. herba laba* and *B. aegyptiaca* before infection, no significant differences were observed in eosinophil counts when compared with the untreated control. The eosinophil counts at week 6 pi was (6%) in *A. herba alba*, (5.5%) in *B. aegyptiaca* and (7%) in the untreated control.

In lambs that were treated with *A. herba alba* and *B. aegyptiaca* post infection, the eosinophil counts were increased after the first week post infection and then they were decreased after the treatment. No significant difference was observed when compared with the untreated control except in week 2 post treatment. The mean eosinophil counts on day 14 post treatment was (3.5%) in *A. herba alaba*, (4.3%) in *B. aegyptiaca* and (3.7%) in albendazole.



1 treated before infection

2 treated post infection

Fig.6. Mean percentage of eosinophils count of experimental lambs

3.1.3.5.2 Lymphocytes Count:

The mean percentages of lymphocyte counts in experimental animals were illustrated in Fig. 7. In all infected groups the lymphocyte counts were increased after the first week post infection with significant differences in weeks 3, 4 and 5 post infection when compared with the uninfected control.

Animals which were treated with *A. herba alba* and *B. aegyptiaca* before infection, showed increase in lymphocyte counts after the first week post infection until week 4 pi without significant differences when compared with the untreated control. The mean of lymphocyte counts at week 6 pi was (66%) in *A. herba alba*, (63.5%) in *B. aegyptiaca* and (64.2) in the untreated control.

In animals that were treated with *A. herba alba* and *B. aegyptiaca* post infection, the lymphocyte counts were increased after the first week post infection until the day of treatment, after that the lymphocyte counts started to decrease after two weeks post treatment. There was no significant difference when compared to the untreated control. The mean percentage of lymphocyte counts on day 14 pt was (64.2%), (64%) and (63.2%) in *A. herba alba*, *B. aegyptiaca* and albendazole respectively.

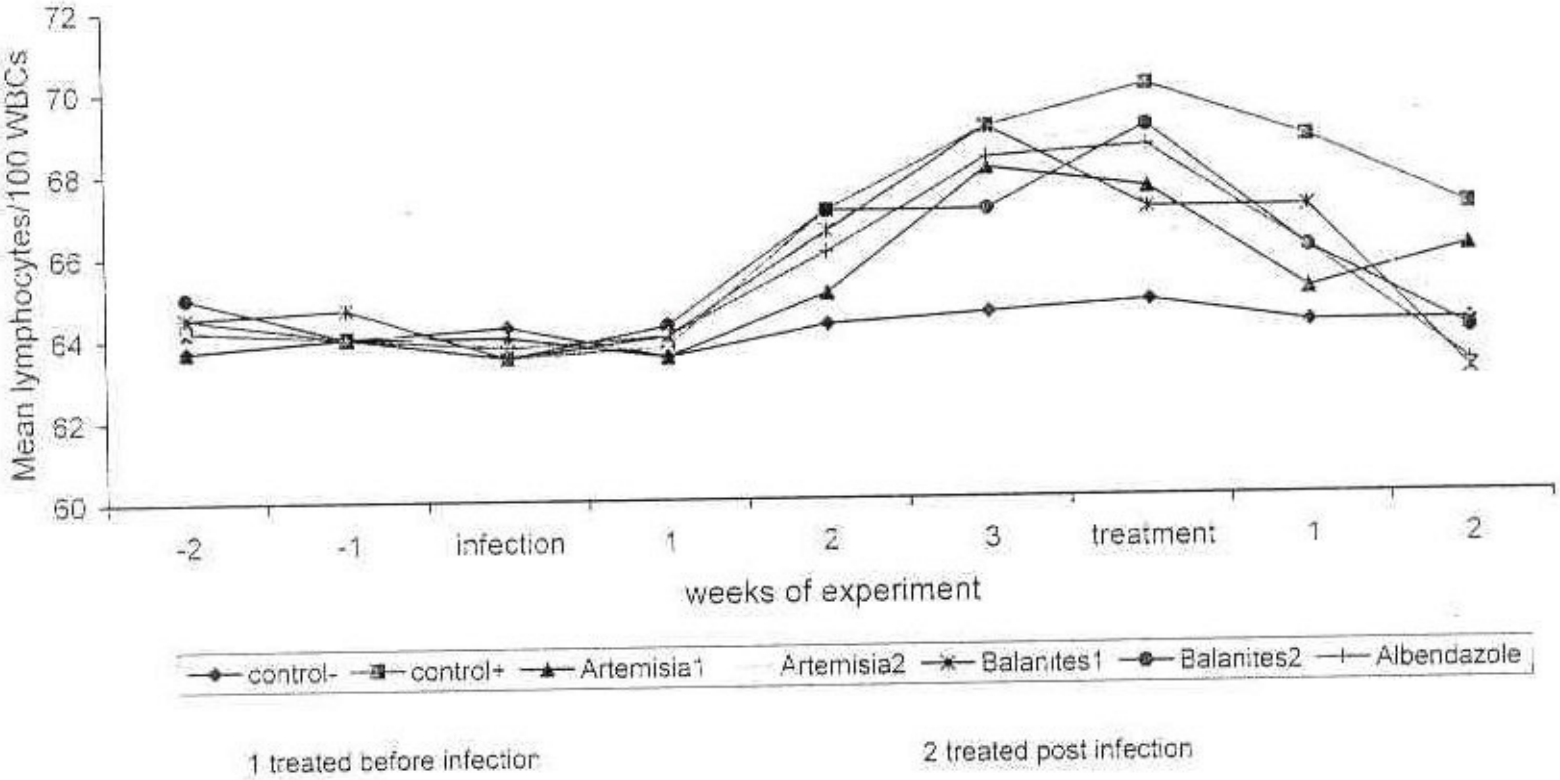


Fig.7. Mean percentage of lymphocytes count of experimental lambs

3.1.3.5.3 Neutrophils Count:

The mean percentages of neutrophil counts in experimental animals were depicted in Fig. 8. All infected animals showed significant decrease in neutrophil counts after the second week post infection when compared with the uninfected control.

Animals treated with *A. herba alba* and *B. aegyptiaca* before infection, didn't show any significant difference in neutrophil counts when compared with the untreated control. The neutrophil counts at week 6 pi of *A. herba alba* and *B. aegyptiaca* was (25%), and (24%) in the untreated control group.

In lambs that were treated post infection with *A. herba alba* and *B. aegyptiaca*, the neutrophil counts showed slight increase after treatment but, without significant difference when compared with the untreated control. The mean percentage of neutrophil counts on day 14 post treatment was (26%) in *A. herba alba* and in *B. aegyptiaca* and (27%) in albendazole.

3.1.4 Serological Findings:

The mean serum constituents (total proteins, albumins, globulins, Sodium, Potassium, Calcium and Phosphorus) of the control animals were fluctuated within the normal range throughout the experimental period. Our

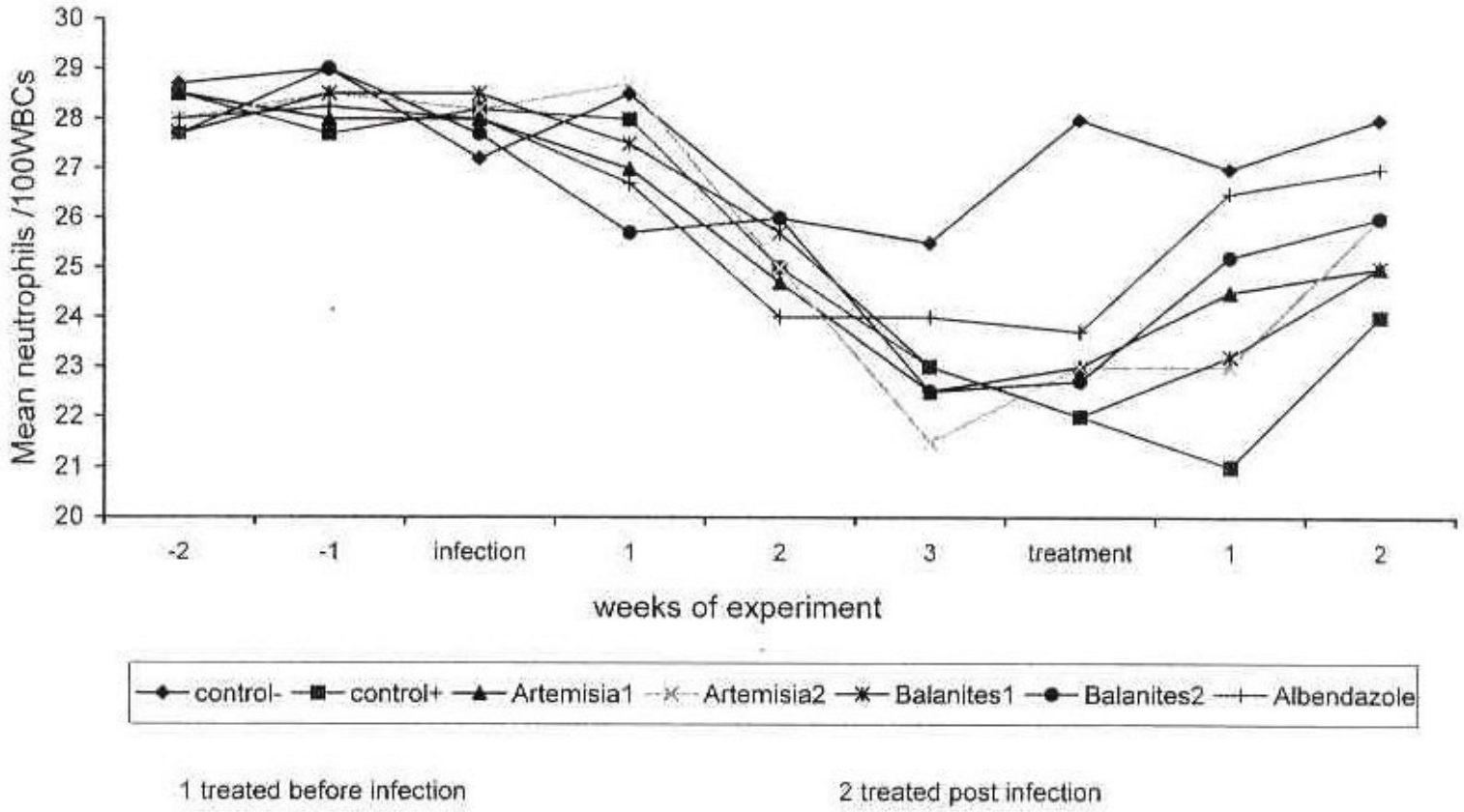


Fig.8. Mean percentage of neutrophils count of experimental lambs

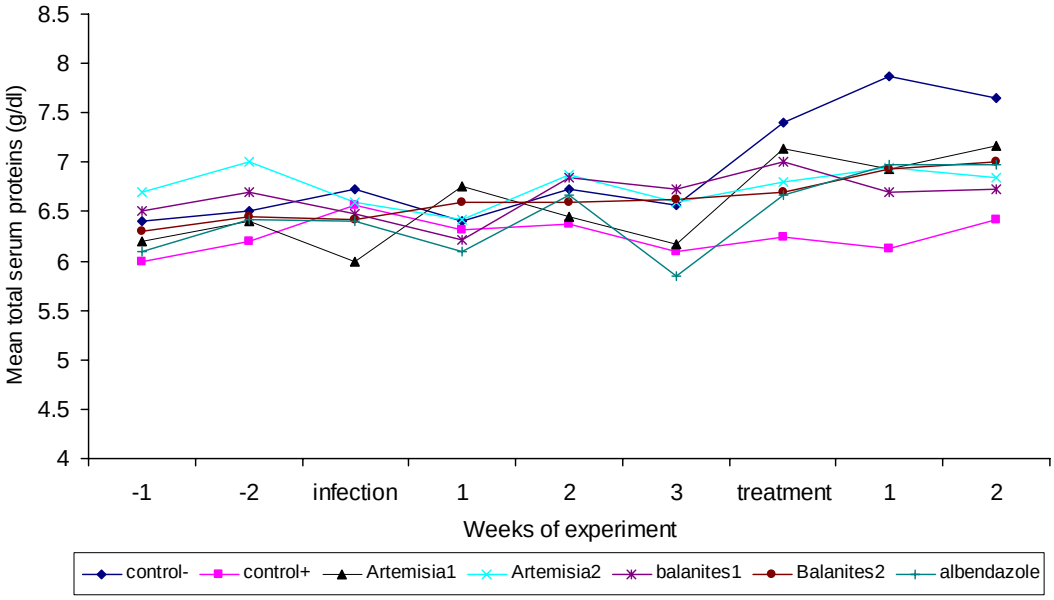
findings related to the Serum constituents in all groups of experimental animals were shown below:

3.1.4.1 Total Serum Proteins:

The mean total serum protein values of experimental lambs were depicted in Fig. 9. All infected lambs showed increase in the total serum protein values at the start of the study, followed by slight fluctuated decrease till the end of the experimental period without significant differences when compared with the uninfected control group.

In lambs which were treated with *A. herba alba* and *B. aegyptiaca* before infection, no significant differences occurred in total serum protein values throughout the experimental period, when compared with the untreated control group. The mean total protein values at week 6pi are (6.7 g/dL) in *B. aegyptiaa,* (7.1 g/dL) in *A. herba alba* and (6.4 g/dL) in the untreated control group.

In animals that were treated with *A. herba alba*, *B. aegyptiaca* and albendazole post infection, no significant differences were observed in total serum protein values (P>0. 05) when compared with the untreated control group, but there was a slight increase in the total serum proteins after the first week post treatment. The mean total serum protein values on day 14 pt are (7 g/dL), (6.85 g/dL) and (6.97) g/dL in *B. aegyptiaca, A. herba alba* and albendazole, respectively.



1 treated before infection

2 treated post infection

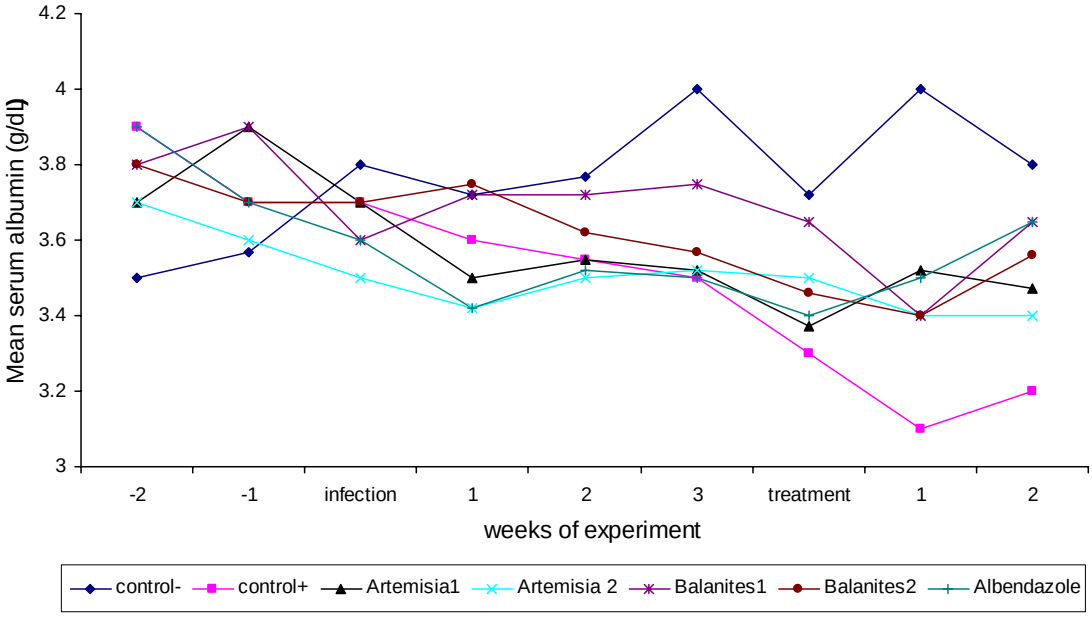
Fig.9. Mean values of total proteins of experimental lambs

3.1.4.2 Serum Albumin:

The mean serum albumin values of experimental animals were illustrated in Fig. 10. The serum albumin values of infected animals fluctuated with a tendency to be lower than that of the uninfected control group.

In lambs which were treated with *A. herba alba* and *B. aegyptiaca* before infection, no significant differences ($P>0.05$) were observed when compared with untreated control group, except in the case of *B. aegyptiaca* which showed significant higher albumin values at week 6 pi. The mean total albumins were (3.7 g/dL), (3.4 g/dL) and (3.2 g/dL) in *B. aegyptiaca, A. herba alba* and the untreated control groups respectively.

On the other hand, lambs that were treated with *A. herba alba*, *B. aegyptiaca* and albendazole post infection, no significant differences were recorded in *B. aegyptiaca* and *A. herba alba* groups. However, albendazole group showed significant higher albumin values ($P<0.05$) at week 6 pi (day 14 pt) when they were compared with the untreated control group. The mean albumin values on day 14 pt were (3.5g/dL), (3.4g/dL) and (3.65g/dL) in *B. aegyptiaca, A. herba alba* and albandzol, respectively.



1 treated before infection

2 treated post infection

Fig.10. Mean serum albumin values of experimental lambs

3.1.4.3 Serum Globulin:

The mean serum globulin values of experimental lambs were depicted in Fig. 11. All infected animals showed a decrease in globulin values after the second week post infection. No significant difference was observed between infected and uninfected control groups; however, in week 5 post infection there was a significant decrease in the group that was treated with *B. aegyptiaca* before infection.

In lambs that were treated with *A. herba alba* and *B. aegyptiaca* before infection, there was no significant difference in globulin values when compared with the untreated control. The globulin values at week 6 post infection were (3.7g/dL) in *A. herba alba*, (2.95g/dL) in *B. aegyptiaca* and (3.27g/dL) in the untreated control.

In animals which were treated post infection with *A. herba alba* and *B. aegyptiaca*, the globulin values were increased after the treatment specially in the case of albendazole group, but this increase was not significant when compared with the untreated control. The globulin values on day 14 post treatment were (3.45g/dL) in *A. herba alba*, (3.37g/dL) in *B. aegyptiaca* and (3.7g/dL) in albendazole.

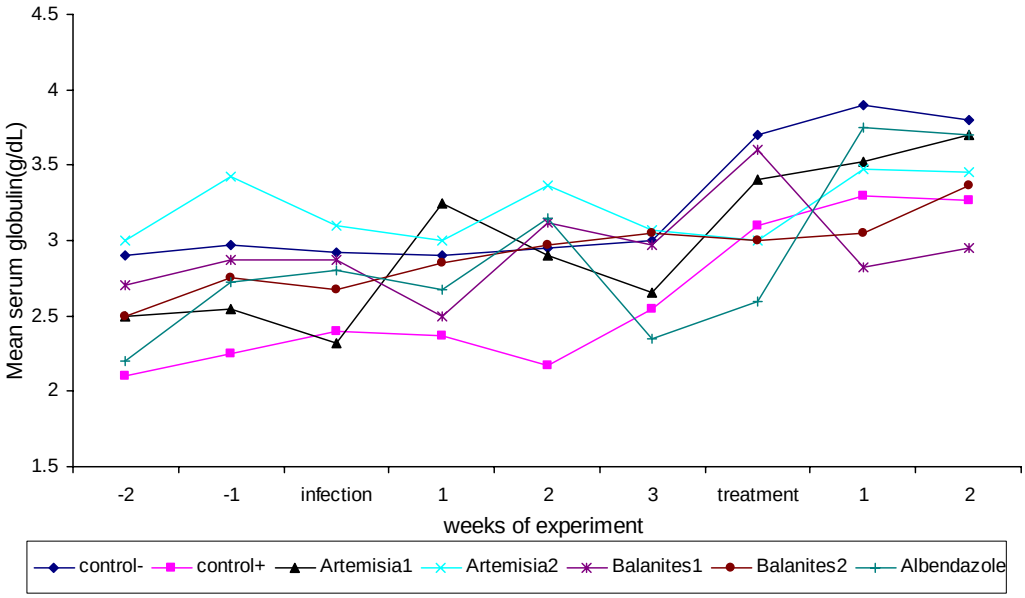


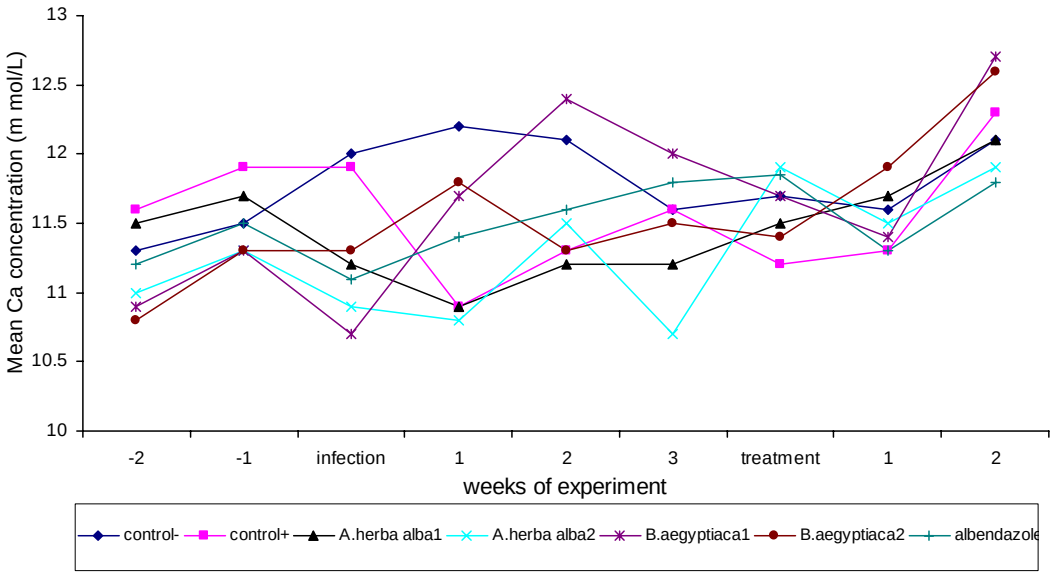
Fig.11. Mean serum globulin values of experimental lambs

3.1.4.4 Calcium:

The mean calcium concentrations of experimental animals were depicted in Fig. 12. Generally the mean calcium concentrations declined in infected lambs when compared with the uninfected control group, but without significant differences.

Animals treated with *A. herba alba* before infection, showed a decline in calcium concentration after infection. However, those treated with *B. aegyptiaca* showed increase in calcium concentration after infection, but without significant difference when compared with untreated control group. The mean calcium concentrations at week 6 pi were (12.6m mol/L) in *B. aegyptiaca*, (12.1 m mol/L) in *A. herba alba* and (12.3 m mol/L) in untreated control.

In animals that were treated with *A. herba alba* and *B. aegyptiaca* after infection, the mean calcium concentrations increased after the first week post treatment; but this increase was not significantly different ($P>0.05$) when compared with the untreated control. The mean calcium concentrations on day 14 pt were (12.6 m mol/L) in *B. aegyptiaca*, (11.9 m mol/L) in *A. herba alba* and (12.8 m mol/L) in albendazole.



1 treated before infection

2 treated post infection

Fig. 12. Mean calcium concentrations of experimental animals

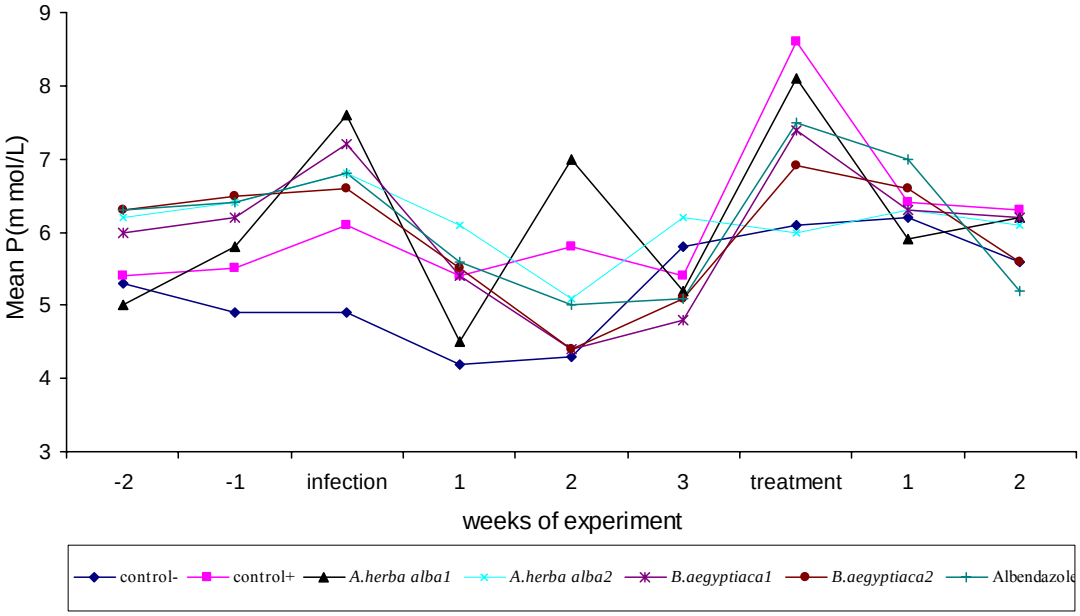
3.1.4.5 Phosphorus:

The mean phosphorus levels of experimental lambs were illustrated in Fig. 13. All infected animals showed insignificant increase in phosphorus levels when compared with the uninfected control group.

Animals that were treated with *A. herba alba* and *B. aegyptiaca* before infection, revealed non significant (P>0.05) decrease in the mean phosphorus concentration when compared with the untreated control group. The mean phosphorus concentrations at week 6 pi were (6.2 m mol/L) in *B. aegyptiaca*, (6.2 m mol/L) in *A. herba alba* and (6.3 m mol/L) in the untreated control group. Likewise, lambs which were treated with *B. aegyptiaca* and *A. herba alba* after infection, revealed insignificant decrease (P<0.05) in the phosphorus levels when compared with the untreated control group. The mean phosphorus concentrations on day14 pt were (5.6 m mol/L) in *B. aegyptiaca*, (6.1 m mol/L) in *A. herba alba* and (5.2 m mol/L) in albendazole.

3.1.4.6 Sodium:

The mean sodium concentrations of experimental lambs were shown in Fig. 14. The mean sodium values of infected animals failed to show consistent pattern of changes throughout the observation period.



1 treated before infection

2 treated post infection

Fig. 13. Mean phosphorus levels of experimental lambs

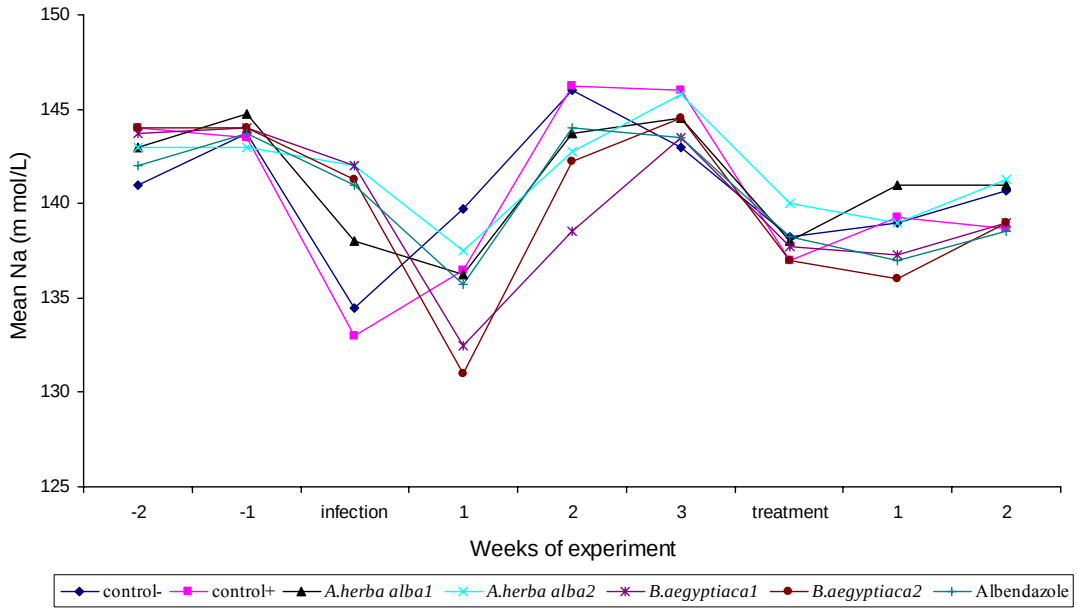
There was no significant difference between infected and the uninfected control lambs.

Animals which were treated with *A. herba alba* and *B. aegyptiaca* before infection didn't show any significant differences when compared with the untreated control group. The mean sodium concentrations at week 6 pi were (139 m mol/L), (141m mol/L) and (138.7 m mol/L) in *B. aegyptiaca*, *A. herba alba* and the untreated control group respectively. Also, no significant differences (P>0.05) were recorded in animals which were treated after infection with *B. aegyptiaca* and *A. herba alba* when compared with the untreated control group. The mean Sodium concentrations on day 14 pt were (139 m mol/L) in *B. aegyptiaca*, (141.2 m mol/L) in *A. herba alba* and (138.5 m mol/L) in albendazole.

3.1.4.7 Potassium:

The mean potassium concentrations of experimental lambs were shown in Fig. 15. Generally the mean potassium values of infected lambs increased after the first week post infection up to the 2nd and forth week pi. However, after that the potassium concentrations returned to the same control values.

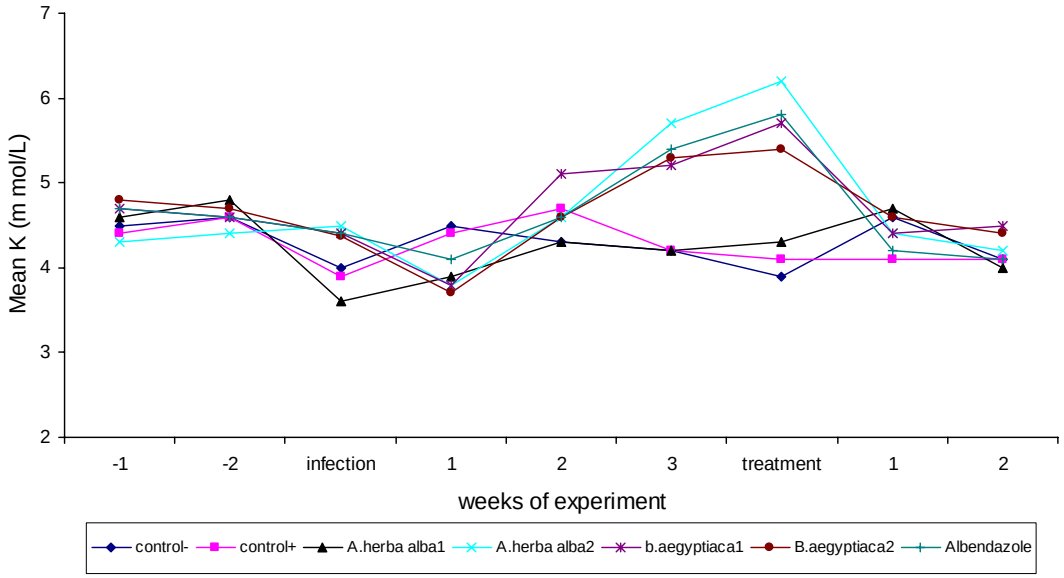
In animals that were treated with *A. herba alba* before infection,



1 treated before infection

2 treated post infection

Fig. 14. Mean sodium values of experimental lambs



1 treated before infection

2 treated post infection

Fig. 15. Mean potassium concentrations of experimental lambs

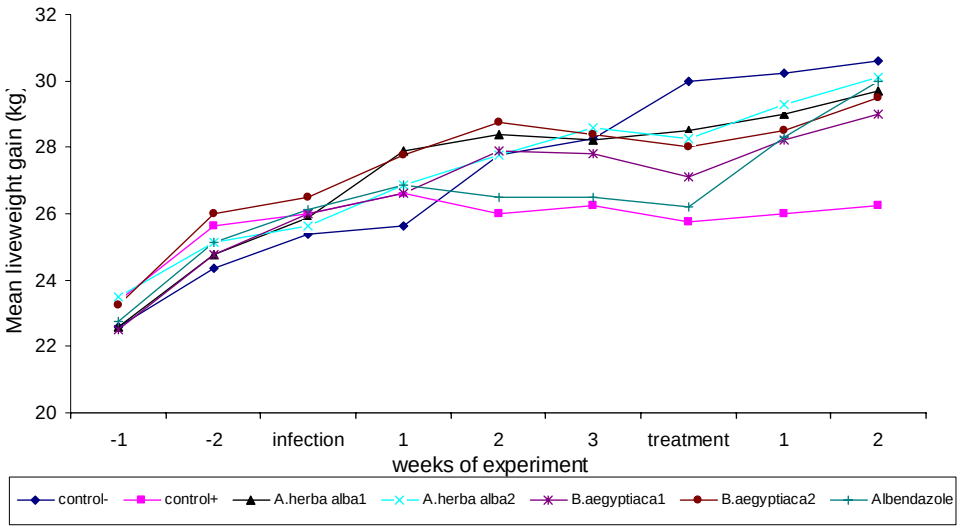
showed very slight increase without any significant difference, but in case of *B. aegyptiaca* there was a significant increase in potassium concentration at week 4 (pi) when compared with the untreated control group. The mean potassium concentrations were (4.5 m mol/L) in *B. aegyptiaca*, (4 m mol/L) in *A. herba alba* and (4.1 m mol/L) in the untreated control group.

In lambs which were treated with *B. aegyptiaca* and A. *herba alba* after infection, no significant differences (P>0.05) were recorded after treatment when compared with the untreated control group. The mean potassium concentrations on day 14 pt were (4.4 mmol/L), (4.2 mmol/L) and (4.1 mmol/L) in *B. aegyptiaca*, *A. herba alba* and albendazole respectively.

3.1.5 Live body weight:

The mean live body weights of experimental lambs were illustrated in Fig. 16. All infected lambs maintained a steady increase in the rate of growth, but generally they showed lower body weight values when compared to the uninfected control. No significant difference was observed between infected and uninfected control groups.

Lambs that were treated with *A. herba alba* and *B. aegyptiaca* before infection showed steady increase in body weight compared with the untreated control but this increase was not significantly different. The mean



1 treated before infection 2 treated post infection

Fig. 16. Mean live weight gain of experimental lambs

live body weights were (29.1 kg), (29.7 kg) and (26.2 kg) in *B. aegyptiaca*, *A. herba alba* and the untreated control respectively.

On the other hand, lambs which were treated with *B. aegyptiaca* and *A. herba alba* post infection, showed slight increase after treatment but this increase was not significantly different when compared with the untreated control group. Also no significant difference was observed in the two medicinal plants (*B. aegyptiaca* and *A. herba alba*) when compared with the albendazole group. The mean live bodyweights on day 14 pt were (29.5 kg), (30.1 kg) and (30 kg) in *B. aegyptiaca*, *A. herba alba* and albendazole respectively.

3.1.6 Pathological findings:

3.1.6.1 Post mortem findings:

In all animals, the general carcass condition was good. No edema or hemorrhages were observed and no salient gross lesions were seen in internal organs except the abomasi which showed mucoid contents which stained dark brown. The mucosa was hyperemic and showed petechial hemorrhages. Adult *H. contortus* worms were recovered from all abomasi.

3.1.6.2 Histopathological finding:

In the infected non-treated lambs the histopathological changes included; dilated gastric glands with widened lamina propria (4/4). Mononuclear cell infiltration, mainly lymphocytes were seen at the basal

region of the gastric mucosa, as small aggregates or as nodules (3/4) (Fig. 17). Scattered lymphocytes were also seen in lamina propria between gastric glands. Eosinophils and neutrophils were seen in the mucosa (3/4) (Fig. 18). In the infected and treated with albendazole group abomasal sections showed mainly mucosal lymphocytic infiltration (3/4), which was seen as large lymphoid nodule in one section involving whole mucosa, and extending in submucosa; prominent mucous cells were also seen(Fig. 19). Paretal and chief cells were prominent. Sections from animals infected with *H. contortus* after receiving *A. herba alba* showed rather dilated and separated gastric glands (3/4). Mucosal lymphocytic infiltration was seen in all animals (4/4) especially at basal layer and slight congestion was also seen (Fig.20). The overall picture resembled that of infected non-treated group but eosinophils and neutrophils were not seen here. Animals infected with *H. contortus* after receiving *B. aegyptiaca* showed similar picture to that of the group treated with *A. herba alba* before infection. In animals treated with *A. herba alba and B. aegyptiaca* after infection, abomasal section showed no prominent pathological changes. In one animal, sections showed prominent mucous cells and lymphocytic infiltration involving mainly the submucosa (Fig. 21).

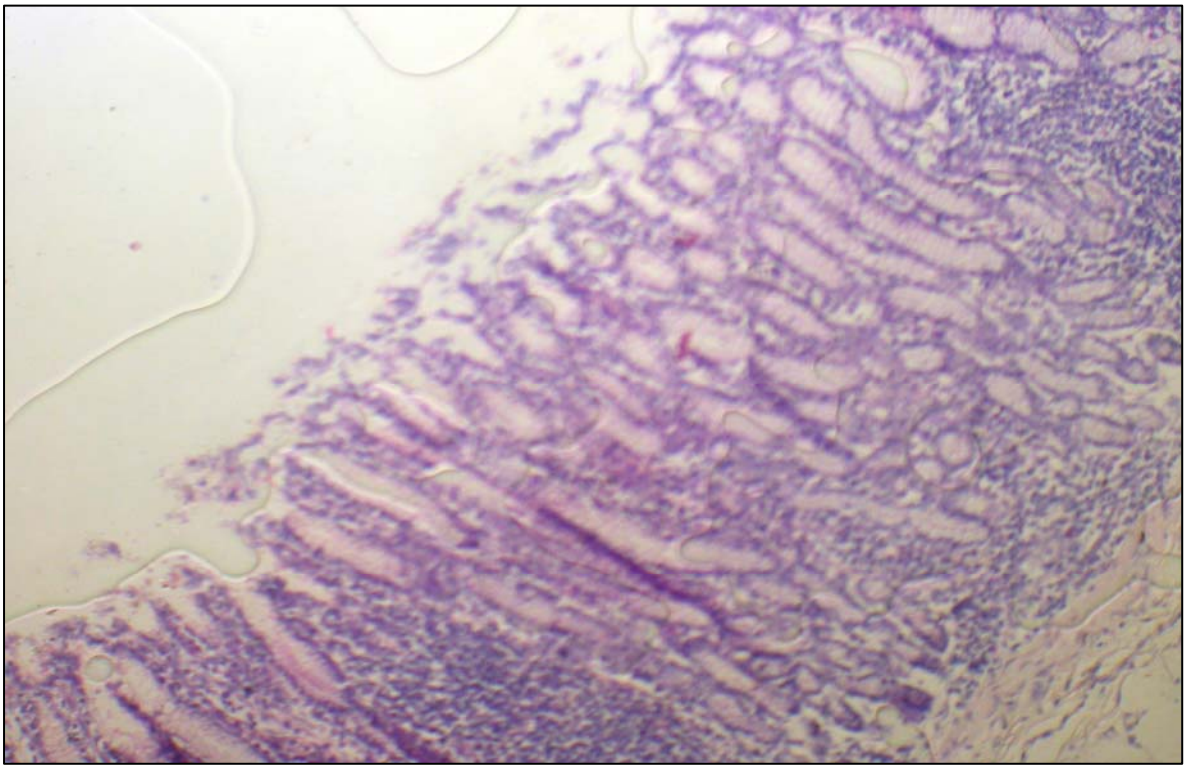


Fig. 17. Abomasal section of infected non treated lamb showed Mononuclear cell infiltration, mainly lymphocytes was seen at the basal region of the gastric mucosa

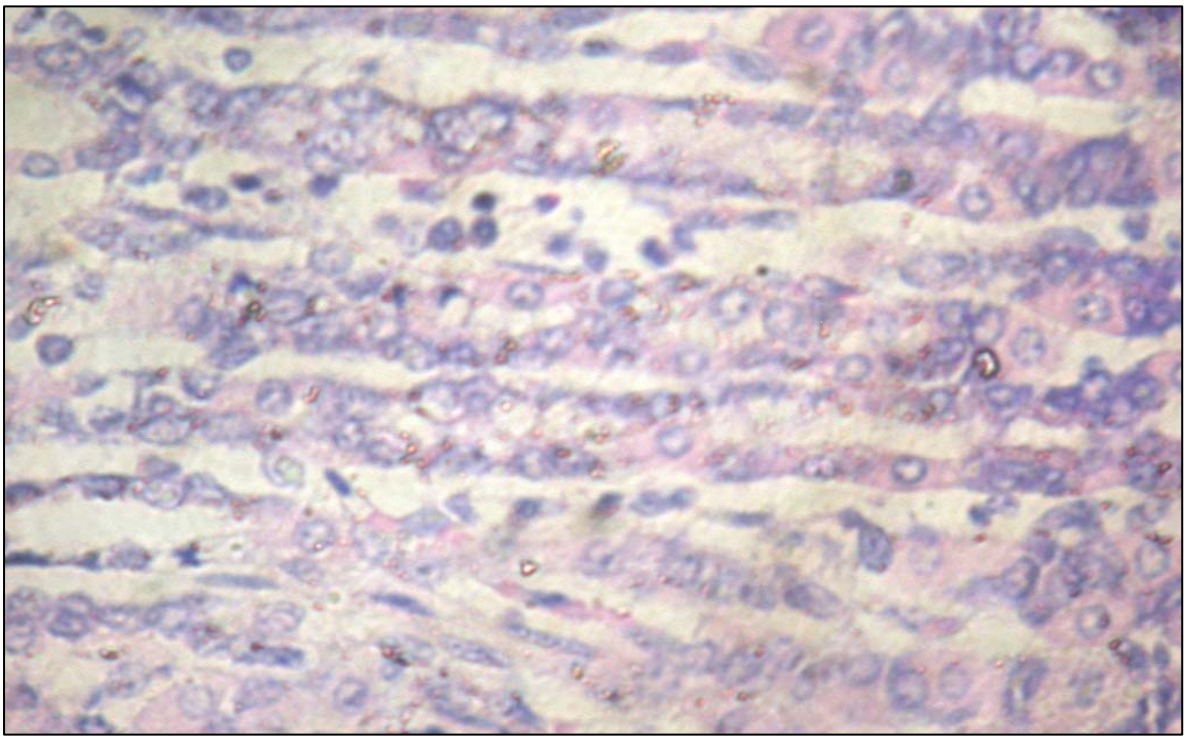


Fig.18. Abomasal section of infected non treated lamb showed eosinophils and neutrophils infiltration in the mucosa

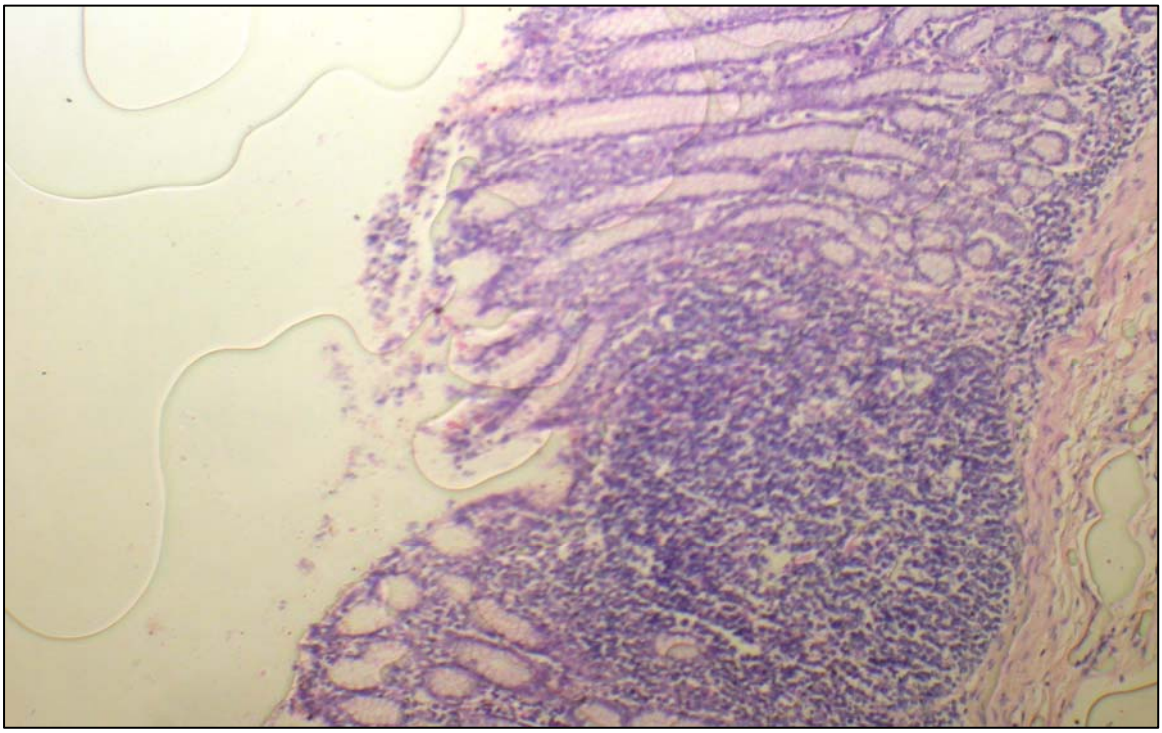


Fig.19. Abomasal section of lambs treated with albendazole, showed mainly mucosal lymphocytic infiltration which was seen as large lymphoid nodule

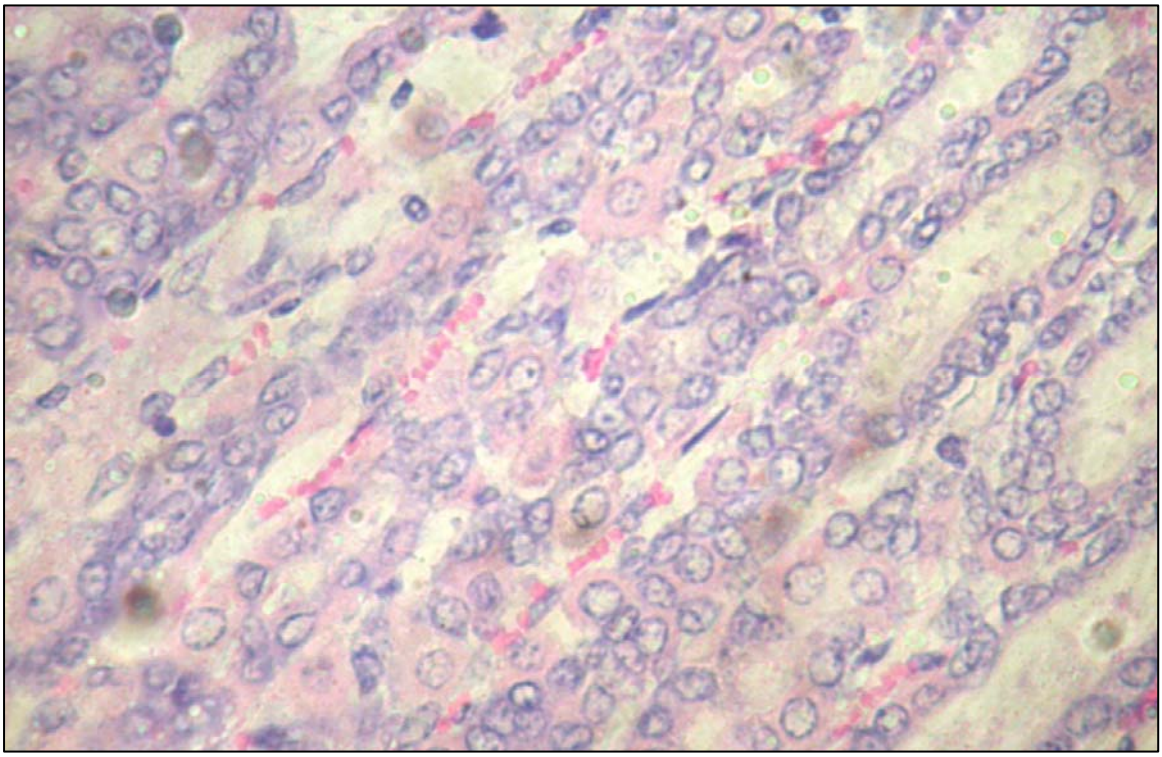


Fig.20. Abomasal section of lambs treated with *A. herba alba* before infection showed slight congestion.

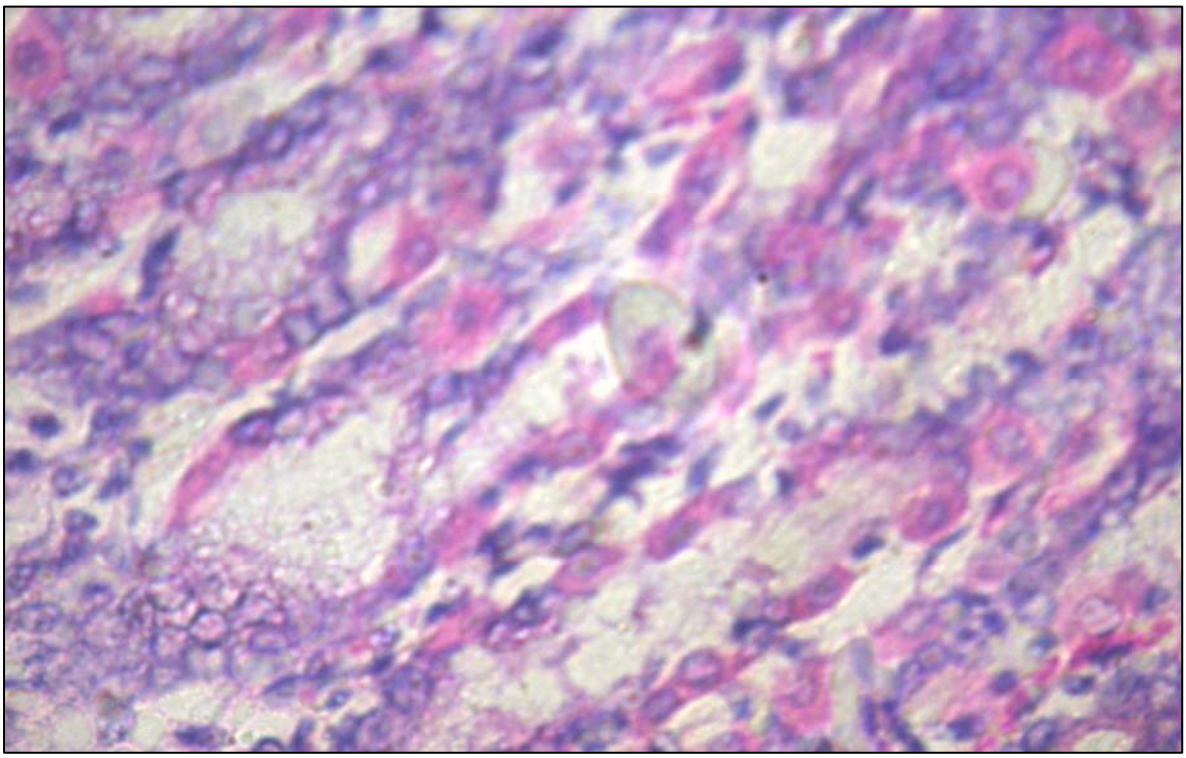


Fig.21. Abomasal section of lambs treated with *B. aegyptiaca* after infection showed prominent mucous cells involving mainly the submucosa

3.2 *In vitro* anthelmintic activity:

3.2.1 Adult motility assay:

The effect of extracts from *A. herba alba* and *B. aegyptiaca* on adult *Haemonchus contortus* worms, expressed as a percentage of dead worms compared to the total number of worms in the Petri dishes, depending on time and concentration of extracts were presented in tables (4 and 5). It is evident from these tables that, the aqueous and methanolic extracts of *A. herba alba* and *B. aegyptiaca* exhibited significant ($P<0.05$) anthelmintic activity against adult *H. contortus* worms as revealed from the death of the worms post exposure to different treatments. The higher doses of both extracts of the plants resulted in an early onset of activity and higher number of dead worms compared with lower doses. These *in vitro* results indicated that, both plants exhibited dose concentration- and time dependent anthelmintic effects by causing mortality of worms.

The worms which were kept as untreated control in PBS did not show any mortality at 6 hours post exposure whereas; the albendazole drug killed all the worms at 2 hours post exposure thus being 100% effective.

Artemisia herba alba:

It is evident from table (4) that, both extracts of *A. herba alba* caused a significant degree of mortality of *H. contortus*. However, the methanol extraction of this plant exhibited greater and quicker anthelmintic activity

in terms of the mortality of the worms than the aqueous extraction using the same concentration. The methanol extraction of *A. herba alba* at 50mg/ml concentration, killed all the worms within two hours post exposure (being 100% effective). All worms were found dead(100% mortality) at 6 hours post exposure to different concentrations of methanol extraction of *A. herba alba*, while the aqueous extraction showed 90% mortality at 50mg/ml after 6 hours post exposure. There was no significant difference between *A. herb alba* methanol extraction and albendazole at 6 hours post exposure.

Balanites aegyptiaca

The anthelmintic effect of *B. aegyptiaca* was shown in table (5). It is evident from this table that, both extracts of *B. aegyptiaca* caused significant levels of mortality of *H. contortus* when compared with the untreated control. However, unlike the case of *A. herba alba*, the aqueous extraction of *B. aegyptiaca* exhibited greater and quicker anthelmintic activity in terms of the mortality of the worms than the methanol extraction at the same concentration. The maximum mortality of the aqueous extraction at concentration of 50mg/ml was 100% after 6 hours post exposure. However, the methanol extraction did not kill all the worms and was found 75% effective at the same concentration after 6h post exposure.

Table (4): *In vitro* effect of aqueous and methanolic extracts of *Artemisia herba alba* on adult *Haemonchus contortus* of sheep

treatments	concentration	Mean number of Mortality percent of worm at Different hours post- exposure			
		1h	2h	4h	6h
A. herba alba water extraction	5 mg/ml	10±5.7 cd	43.3±3.3 cd	56.7±3.3e	63.3±3.3 c
	10 mg/ml	20±5.7 c	43.3±6.6 cd	60.3±3.3 de	73.3±3.3 c
	25 mg/ml	23.3±3.3 c	50±5.7 b	73.3±3.3 d	80±0 b
	50 mg/ml	40±11.4 b	70±3.3 b	76.7±5.7 cd	90±0 a
A. herba alba methanol extraction	5 mg/ml	3.3±3.3 d	30±5.7 d	63.3±8.8 d	100±0 a
	10mg/ml	30±5.7 bc	60±5.7 bc	80±5.7 bc	100±0 a
	25mg/ml	63.3±3.3 a	80±5.7 b	96.7±3.3 ab	100±0 a
	50mg/ml	73.3±3.3 a	100±0 a	100±0 a	100±0 a
PBS		0 ±0 d	0±0e	0±0 f	0±0 d
Albendazole	25mg/ml	80 ±5.7 a	100 ±0 a	100±0 a	100±0 a

Means (±SE) followed by the same letter in each column are not significantly different at 5% level based on REGWQ.

Table (5): *In vitro* effect of aqueous and methanolic extracts of *Balanites aegyptiaca* on adult *Haemonchus contortus* of sheep

treatments	concentration	Mean number of Mortality percent of worm at Different hours post- exposure			
		1h	2h	4h	6h
B. aegyptiaca water extraction	5 mg/ml	0±0 d	13.3±6.6 ed	46.6±8.8 c	73.3±6.6 b
	10 mg/ml	13.3±3.3 cd	30±5.7 cd	50±5.7 c	76.7±3.3 b
	25 mg/ml	30±5.7 c	40±5.7 c	60±0 bc	80±5.7 ab
	50 mg/ml	53±12 b	63.3±3.3b	83.3±3.3 ab	100±0 a
B. aegyptiaca methanol extraction	5 mg/ml	0±0 d	0±0 e	36.6±3.3 c	40±3.3 c
	10mg/ml	0±0 d	10±5.7de	40±10 c	50±5.7 c
	25mg/ml	3.3±3.3 d	13.3±3.3de	50±10 c	60±5.7 bc
	50mg/ml	3.3±3.3 d	16.7±6.6de	60.3±8.8 bc	75±3.3 b
PBS		0±0d	0±o e	0±0 d	0±0 d
Albendazole	25mg/ml	80±5.7a	100±o a	100±0 a	100±0 a

Means (±SE) followed by the same letter in each column are not significantly different at 5% level based on REGWQ.

3.2.2 Larvicidal anthelmintic activity:

The results of the *in vitro* determination of the activity of methanol and aqueous extraction of *A. herba alba* and *Balanites aegyptiaca* against third larvae of *H. contortus* are summarized in tables (6 and 7). It is evident from these tables that, all foresaid treatments irrespective of the concentration and time post exposure had no effect on the motility of the third larval stage. There was no significant difference in the effect of motility of third larval stage between both types of extraction of *A. herba alba* and *B. aegyptiaca* in relation to albendazole and the control.

Table (6): *In vitro* motility effect of aqueous and methanolic extracts of *Artemisia herba alba* on third larval stage of *H. contortus*

treatments	concentration	Mean number of Mortality percent of larvae at Different hours post- exposure			
		2h	6h	12h	24h
A. herba alba water extraction	5 mg/ml	0	0	3.3	0
	10 mg/ml	3.3	0	0	0
	25 mg/ml	0	0	0	6.6
	50 mg/ml	3.3	3.3	0	0
A. herba alba methanol extraction	5 mg/ml	0	3.3	3.3	0
	10mg/ml	0	0	0	0
	25mg/ml	0	3.3	0	0
	50mg/ml	3.3	0	0	0
water		0	0	0	0
Albendazole	25mg/ml	0	3.3	0	10

Table (7): *In vitro* motility effect of aqueous and methanolic extracts of *Balanites aegyptiaca* on third larval stage of *H. contortus*

treatments	concentration	Mean number of Mortality percent of larvae at Different hours post- exposure			
		2h	6h	12h	24h
B. aegyptiaca water extraction	5 mg/ml	0	0	0	0
	10 mg/ml	0	0	3.3	0
	25 mg/ml	0	0	0	3.3
	50 mg/ml	0	3.3	0	3.3
B. aegyptiaca methanol extraction	5 mg/ml	0	0	3.6	4
	10mg/ml	0	0	0	0
	25mg/ml	0	0	3.3	3.3
	50mg/ml	0	0	0	0
water		0	0	0	0
Albendazole	25mg/ml	0	3.3	0	10

3.2.3 Transmission Electron Microscopic observations:

3.2.3.1 Control worms:

Histologically, the body of the nematode is covered with a thin tegument.The tegumental muscle showed compact muscle fiber and groups of mitochondria are seen at the base of the muscle fibers (Fig. 22). The intestines of untreated *H .contortus* showed large numbers of mitochondria scattered in the cytoplasm. The microvilli were adjacent, regular in size and the nucleus appeared normal (Fig.23). The uterus, of the untreated worms showed normal appearance of the veteline glands (Fig. 24).

3.2.3.2 Treated worms:

At histological and ultrastructural levels, changes were observable in tegumental muscles, digestive tract (intestines) and the reproductive system (uterus).

Tegumental muscle of *H. contortus* after treatment with *A. herba alba* was shown in (Fig. 25). The mitochondria were seen at the base of muscle fibers as elongated structures; some were small and others were swollen. The muscle fibers were hypertrophied compared with the untreated control worms (Fig. 22).

The intestines of *H. contortus* after treatment with *B. aegyptiaca* were shown in Figs (26. a, b). The microvilli were spaced, some were lost,

others were irregular and were reduced in size when compared with the untreated control (Fig 26a).the reticulum in the cytoplasm was convoluted in the form of concentric formations looking- like finger prints in the rough endoplasmic reticulum. There were intense vaculations of some intestinal epithelial cells (Fig. 26b). The intestines of *H. Contortus* following exposure to *A. herba alba*, showed vesicle formations, misshapen mitochondria and large autophagic vacuoles in the intestinal cells (Fig. 27). The intestines of the worms treated with albendazole showed disrupted mitochondrial walls and large numbers of autophagic vacuoles were observed (Fig. 28).The uterus of *H. contortus* after treatment with both *A. herba alba*, and *B.aegyptiaca* (Figs.29,30) showed sever distortion with disorganization of the vetelline glands. The autophagic vacuoles with a lipid droplets showed disrupted veteline glands, some of these droplets appeared to replace the veteline globules.

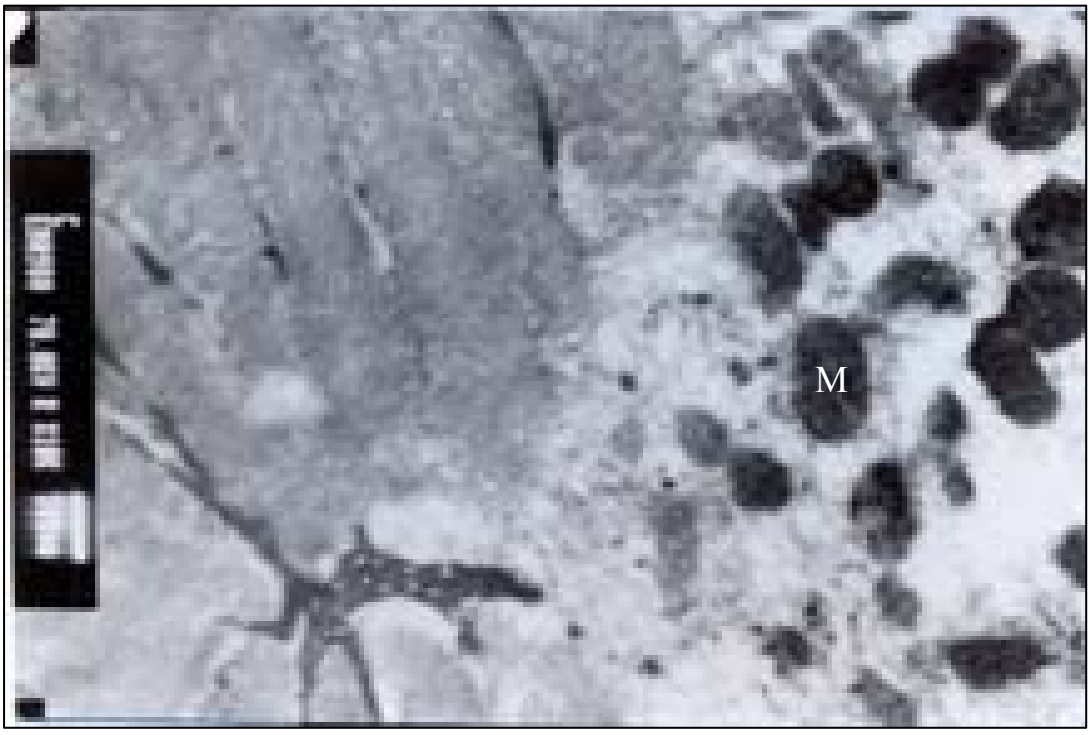


Fig (22).Tegumental muscle of control worm

Showed compact muscle fibers, mitochondria (M) are shown at the base of muscle fiber

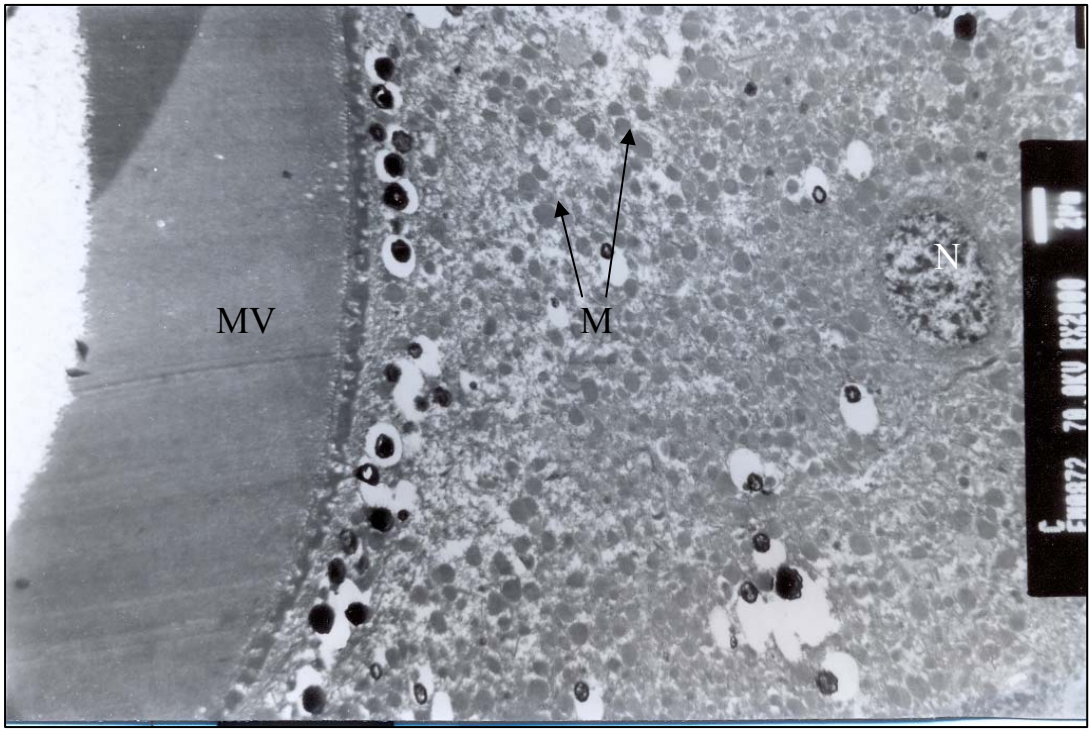


Fig. (23). The intestines of untreated *H. contortus*

Showed large numbers of mitochondria (M), the microvilli were adjacent, regular in size (MV), the nucleus (N) appeared normal

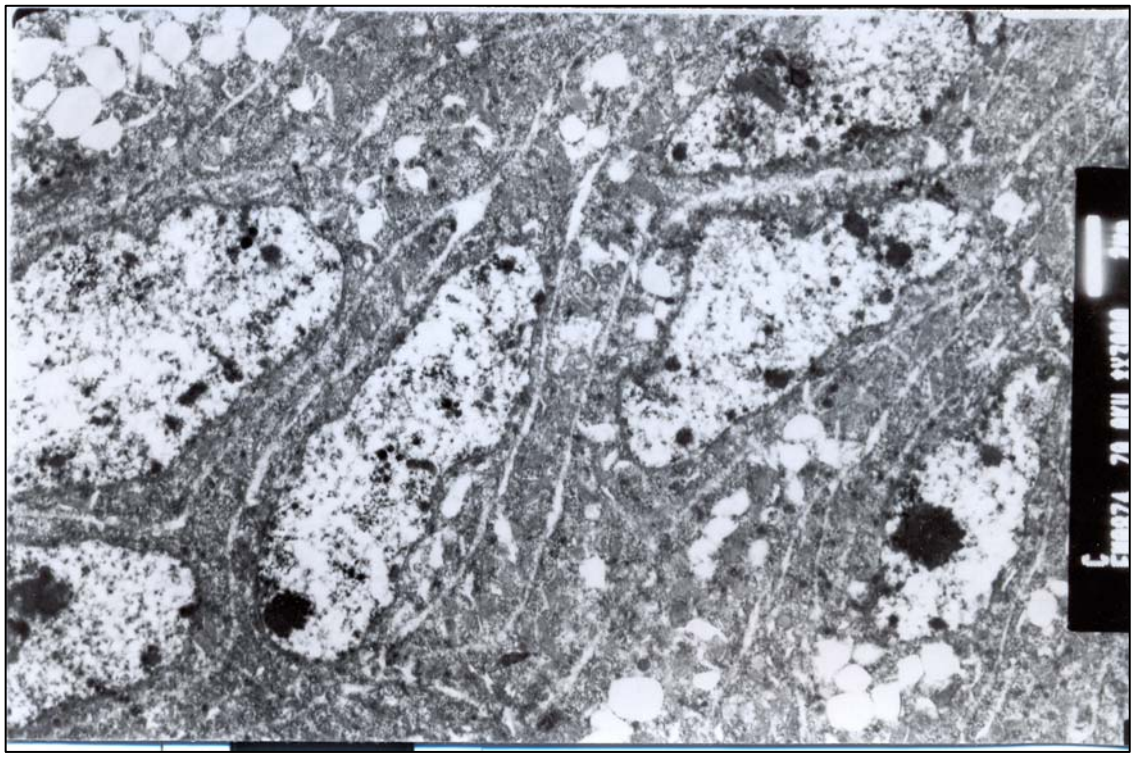


Fig. (24). The uterus of untreated worm
Showed vetelline glands

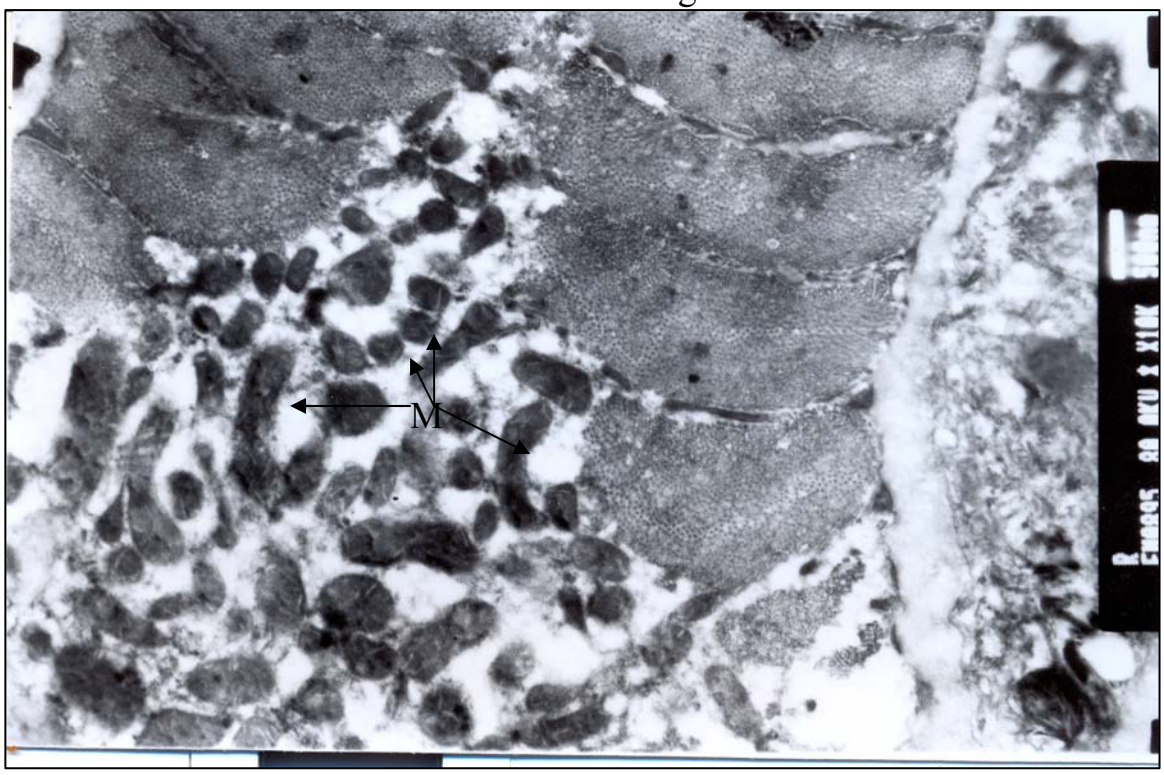


Fig. (25). Tegumental muscle of *H. contortus* after treatment with *A. herba alba,* The mitochondria (M) were elongated; some were small and others were swollen. The muscle fibers were hypertrophid.

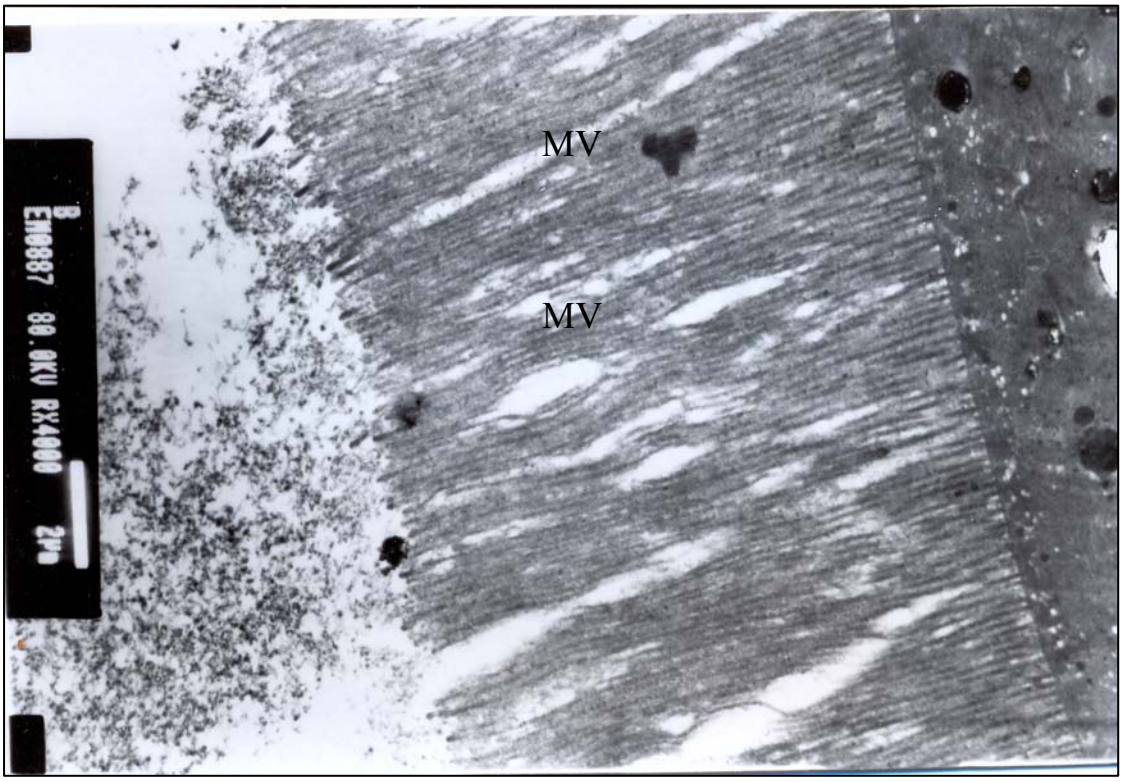


Fig. (26a). The intestines of *H. contortus* after treatment with *B. aegyptiaca* The microvilli(mv) were spaced; some were lost, irregular and reduced in size.

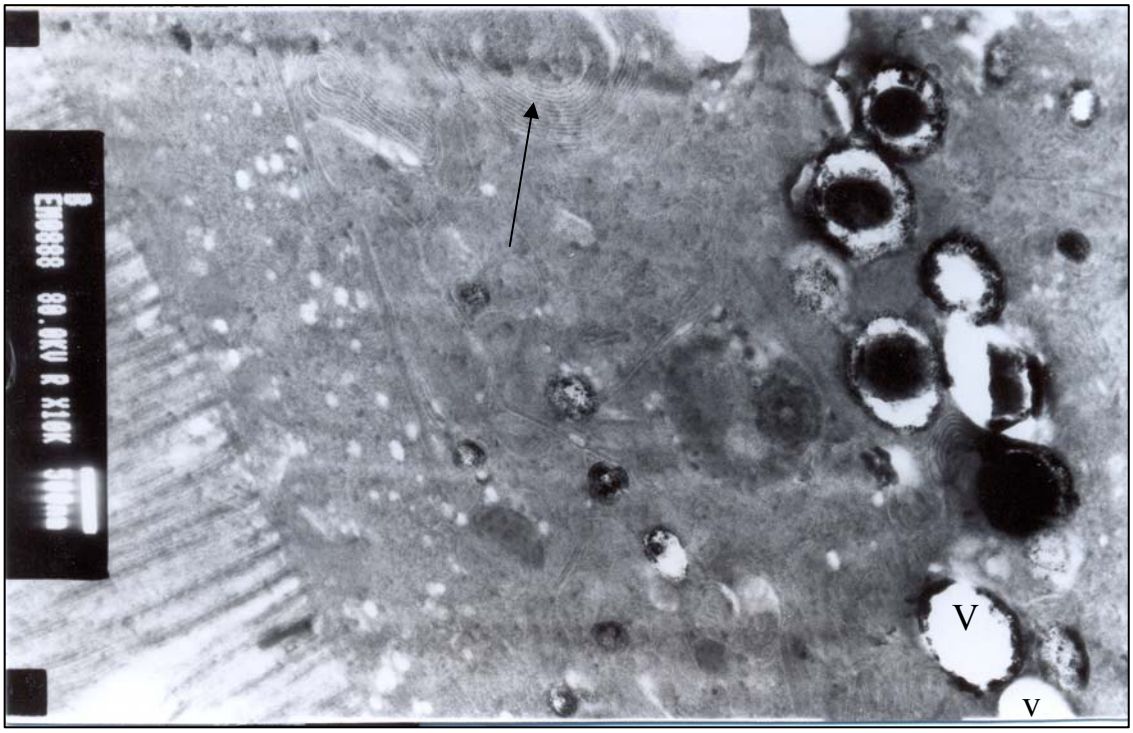


Fig. (25b). The intestines of *H. contortus* after treatment with *B. aegyptiaca* the reticulum in the cytoplasm was convoluted in the form of concentric formations looking- like finger prints(→), There were intense vaculations (V) of some intestinal epithelial cells.

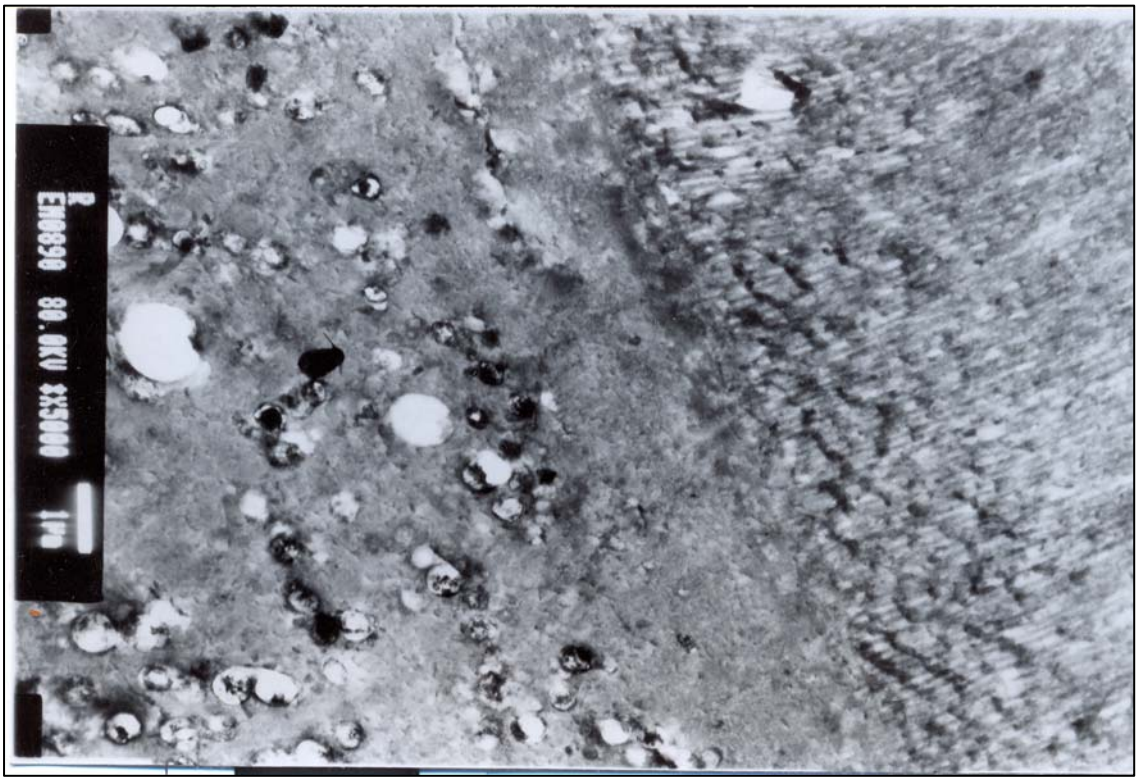


Fig. (27). The intestines of *H. contortus* after treatment with *A. herba alba*
Showed misshapen mitochondria and large autovagic vacules(V)

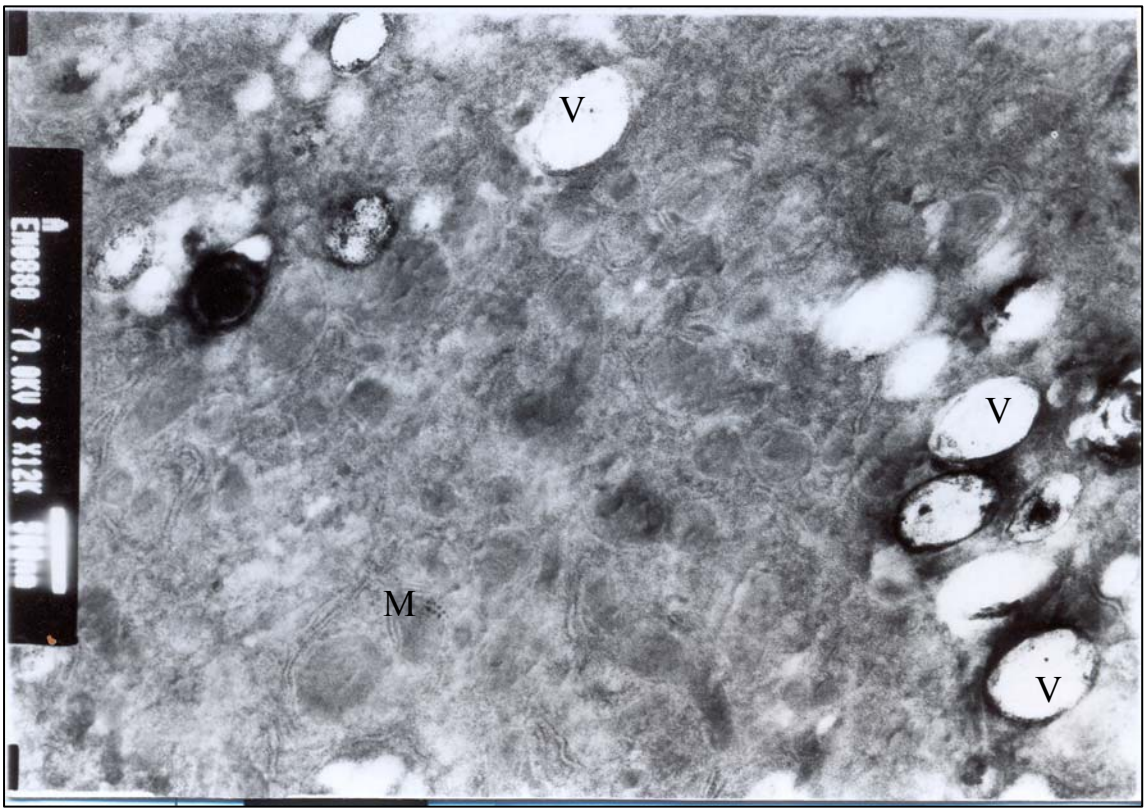


Fig. (28). The intestines of *H. contortus* treated with albendazole
Showed disrupted mitochondrial wall (M) and autophagic vacules (V)

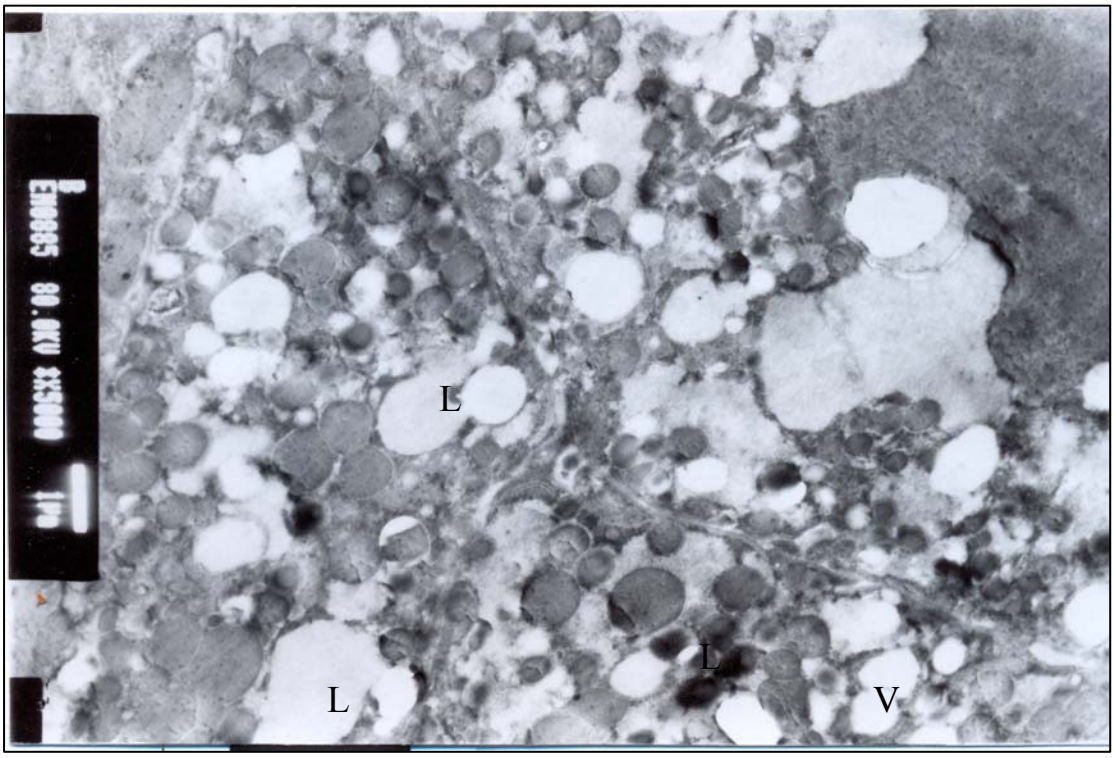


Fig. (29). The uterus of worm treated with *B. aegyptiaca*

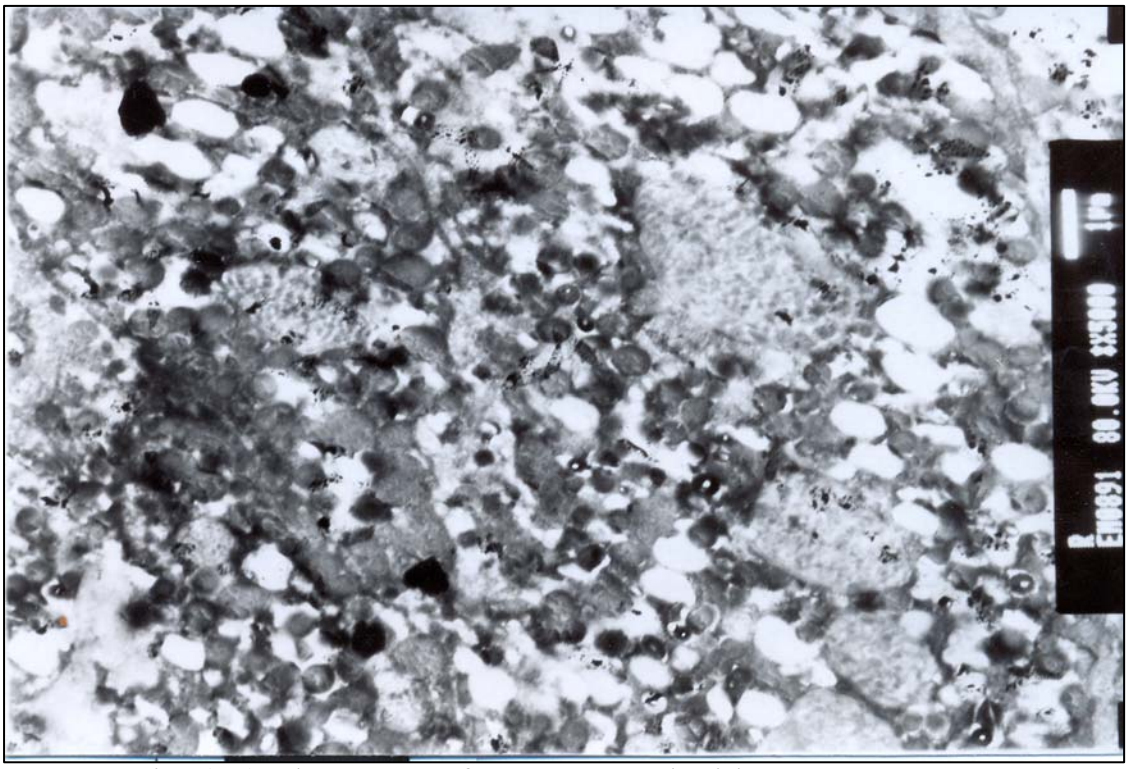


Fig. (30).The uterus of worm treated with *A. herba alba*

Figs. (29, 30) The uterus of worm treated with *B. aegyptiaca and A. herba alba*, showed sever distortion with disorganization of the vetelline glands. The autophagic vacuoles (V) with lipid droplets (L) showed disrupted veteline glands, some of these droplets appeared to replace the veteline globules.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

In recent years, in the Sudan, there has been an increasing interest in sheep industry and breeding for export purposes. The export of sheep and mutton is an important source of foreign currency earning and thus a vital commodity in the Sudan economy. Various disease problems face sheep industry in the Sudan. Infection with gastrointestinal nematodes (GIN), particularly *Haemanchus contortus* continued to be the most important constraint to profitable small ruminant production. Control of *H. contortus* often relies on the use of anthelmintic drugs; widespread intensive use or sometimes poor quality drugs (Monteiro *et al*., 1998) led to development of a high level of multiple anthelmintic resistance in many parts of the developing world (Waller, 1997). This emerging challenge has greatly increased pressure to find alternative, non-chemical control methods of small ruminant GIN, as replacement/supplement to the exclusive use of chemical anthelmintics. The plants are known to provide a rich source of botanical anthelmintics (Satyavati *et al*., 1976). A number of Sudanese medicinal plants have been used to treat parasitic infections in man and animals (Galal *et al*., 1991; Koko *et al*., 2005). The present study was carried out to evaluate the *in vivo* and *in vitro* anthelmintic effects of *A.*

herba alba and *B. aegyptiace* against *H. contortus* in sheep compared with the common commercial drug albendazole.

The *In vivo* studies are more relevant in control practices of gastrointestinal nematodes in farm animals and thus considered more reliable than *in vitro* studies in evaluation of plant extract properties, although costs of large scale screening of plant extract is probably inhibitory (Githiori *et al*., 2006). The *in vivo* studies normally have parasitized hosts being treated with known quantities of plant products and compared with untreated control or with commercial standard anthelmintic (AlShaibani *et al*., 2009). The *in vivo* results indicated that, all infected lambs showed clinical sings of haemonchosis which were manifested by inappetant, dullness, slight reduction in body weight. This might be due to the dependence of severity of disease on the number of infective larvae given to the recipient animals (Rahman and Collins, 1990). No abnormal behavioral change and no evidence of toxicity were recorded during or after the treatment, similarly to those observed by Githiori *et al*., 2004; Igbal *et al*. 2006; Alshaibani *et al*., 2009). The prepatent period of *H. contortus* in this study was 17 days in all infected groups either treated before or after infection or not treated. A finding suggesting that, the *B. aegyptiaca* and *A. herbat alba* didn't show any effect on development of larvae when dosed before infection. Thus, this is the first report of

prophylactic anthelmintic effect of medicinal plant in livestock of Sudan. Animals treated with *B. aegyptiaca* and *A. herba alba* before infection showed no significant reduction in EPG counting when compared with the untreated control. These results indicated that, the *B. aegyptiaca* and *A. herba alba* have no prophylactic effects when treated before infection. This may be due to the ineffectiveness of these plants on the larvae of *H. contortus*, or the quicker withdrawn of plant extracts from the host than the maturation of the larval stages in the abomasi.

The total worm burden at necropsy indicated that, the Hamari breed employed in this study was susceptible to experimental infection with *H. contortus*. However, the establishment percentage was 25%. These findings were confirmed by previous results on susceptibility of Sudanese sheep breed to experimental infection of *H. contortus*; Elhassan, (2002) found that the establishment of Hamari breed was 24.6%. In the present study, animals treated before infection showed reduction in worm count but this was not significant when compared with the untreated control. Different hypotheses can explain the lack of *in vivo* prophylactic activity of *A. herba alba* and *B. aegyptiaca* extracts such as: biotransformation of active substances of the extract, the extract dose or duration of treatment (Hennesy, 1997). Also these plants were not effective against larval stages of *H. contortus*. The lack of the effect of quebracho condensed tannins on

H. contortus larvae was also reported by Paolini (2005). Similarly, in sheep infected with *Teladorsagia circumcincta* and *T. vitrinus*, the consumption of fresh sainfoin before infection did not provoke any changes in worm establishment compared to the control group (Thamsborg *et al*., 2003).

In this study, *Aremisia herba alba* water extract and *B. aegyptiaca* fruit mesocarp exhibited anthelmintic effect when compared with the untreated control group. Similar results were obtained previously by many workers (Akhtar and Riffat, 1985; Hördegan *et al*., 2003; Kahiya *et al*., 2003; Igbal *et al*., 2005; Igbal *et al*., 2006a, b; Jabbar *et al*., 2007; Ademola *et al*., 2007; Tadesse *et al*., 2009). The treatment of *H. contortus* with *B. aegyptiaca* fruit mesocarp at a dose of 9 g/kg showed anthelmintic efficacy of 69.9% and maximum reduction of 69.6% in EPG at day14 post treatment. Similar results were found by Koko *et al*. (2005) and Ibrahim (1992) who reported that, the efficacy percentage of the treatment of *B. aegyptiacai* against *Coenorhabditis elegans* was 45-100%. Unlike in a previous study, where the efficacy of *B. aegyptiaca* was found to be 92.3% against *F. hepatica* (Koko *et al*., 2000). This indicates that the anthelmintic efficacy of the plants differs with respect to different groups of helminthic parasites (Carolin komen *et al*., 2005). The possible explanation of these results was that, the *Balanites aegyptiaca* has been

reported to show a wide range of biological activities which were mainly attributable to its saponin constituents (Eskander, 1982).

In the present study the treatment of *H. contortus* with *A. herba alba* water extracts showed anthelmintic efficacy of 62.5 % and maximum reduction in EPG of 61 % at day14 post treatment. The anthelmintic activity of *Artemisia* species may be attributable to its santonin content responsible for purgative or stimulatory effects on the stomach, nervous and circulatory systems (Watt and Breyer-Brandwijk, 1962). These results support the finding of Idris *et al*. (1982) who reported efficacy of 66.6% against experimental infection of *H. conortus* in goats fed with 30 g/kg of *Artemisia herba alba* shoot. Anthelmintic activity of various species of *Artemisia* had been reported against *Ascaris, Trichostrongylus* and *Strongyloides* (Narayana *et al*., 1976; Nakhare and Garag, 1991; Sharma, 1993). Similar results were obtained by Igbal *et al*. (2004) who found 67.2% reduction in EPG of *H. conortus* at day 14 post treatment in sheep treated with 3 g/kg of *Artemisia brevifolia* aqueous extract, while Tariq *et al*. (2009) found 80.4% reduction in epg count of *H. contortus* treated with 2 g/kg of *Artemisia absinthium*. However, these results seem to be in contrast to those reported by Githiori *et al*. (2004) and Githiori *et al*. (2002); their results showed that, the extracts of the *Myrsine africana* and *Rapanea melanophloeos* were not efficacious against *H. contortus* in sheep.

Also these results were contrary to the results of Ketzis *et al*., (2002) and Costa *et al*. (2006) who showed that, non of faecal egg counts and worm burden of animals treated with *Chenopodium ambrosioicks* and *Azadirachta indica* was statistically different when compared with the untreated control group. Also Bizimenyera *et al*. (2008) reported no significant reduction in EPG of sheep treated with *Peltophorum africa*na.

In this study the treatment of *H. contortus* with 5 mg/kg of albendazole showed anthelmintic efficacy of 97.4%. The albendazole acted well against mature stages of the parasite and the drug was responsible for delaying some worms from reaching their maturity, as was shown at necropsy. In these results, *B. aegyptiaca* and *A. herba alba* extracts showed 69.9 and 62.5% efficacy; the doses used to obtain these results were 9 and 3 g/kg respectively compared with albendazole (5 mg/kg) which was used as a positive control. This fact can be explained by the presence of small concentrations of the active ingredients in the plant extracts (Rates, 2001). Unlike synthetic anthelmintics; where the chemical compounds are isolated in pure form.

In the present study all infected lambs showed slight decrease in PCV values, Hb concentration and erythrocyte counts when compared with the uninfected control. These decreases might be attributable to the blood loss that resulted partly from sucking activity of both larval and adult stages

from hemorrhages associated with the damaged epithelium of the abomasi (Rahman and Colins, 1990). Lambs treated with both *B. aegyptiaca* and *A. herba alba* before infection didn't show significant increase in PCV, RBC or Hb when compared with the untreated control, probably because the reduction in worm burden was low. Each worm is responsible for daily loss of about 0.05ml of blood through ingestion and seepage from lesions (Urquhart *et al*., 1996).

In animals treated after infection there was a progressive reduction in PCV, haemoglobin concentration and total RBCs from the 2nd week post infection; significant increase in haematological parameters was observed after two weeks post treatment, and this may indicate the improvement in the condition of the animal treated after the reduction in the number of worms. Similar results were obtained by Koko *et al*. (2000); Terrill *et al*. (2007); Adam (2006). These results disagreed with Costa (2006); Eguale *et al*. (2007) and Bizimenyera *et al*. (2008), who showed that, treatment of animals with our medicinal plants did not help the animals to improve or maintain their haematological parameters.

In this study the insignificant increase in leukocyte counts of infected lambs was a reflection of insignificant increase in lymphocytes and eosinophils at the expense of neutrophils, which showed reduced counts than the normal levels. Similar results were reported after experimental *H*.

contortus infection by Elhassan (2002). The increased number of leukocytes could be related to an antigenic stimulation of abomasal mucosa that induced proliferation or differentiation of leukocytes. The increase in the number of eosinophils is a common feature observed during *H. contortus* infection (Salamn and Duncan, 1984). No significant differences were observed in leukocytes count, eosinophils, lymphocytes and neutrophils in animals treated either before or after infection, but there was some reduction recorded at the last two weeks in all treated groups. Similar results were observed by Koko *et al*., (2000) who reported the reduction in WBCs and eosinophiles count at the last 2 weeks post treatment.

In the present work the serum protein analysis indicated a decreased total serum protein, albumin and globulin in lambs of all infected groups. The reduction in serum albumin may be related to the direct blood loss inflicted by the sucking activities of both larvae and adult of *H. Contortus*, or due to the bleeding that resulted from damage or ulcerations of the abomasal mucosa. Reduction in the serum proteins have been reported in sheep during *H. Contortus* infection (Rahman and Collins, 1990). The total proteins and albumins had increased progressively in treated animals two weeks after treatment; this indicated an improvement due to the treatment and reduction of worm burden. These results were found to be similar to those reported by Koko *et al*. (2000) and Adam (2006).

Analysis of the mineral contents of the serum showed reduced calcium levels in all infected groups. Serum potassium and phosphorus levels showed insignificant increase, however sodium failed to show consistent pattern throughout the experimental period. The decrease in the level of serum calcium may be related to indigestion of calcium in the abomasi. Most serum calcium is bound to plasma protein; the resulted hypocalcaemia may also be related to the reported hypoproteinaemia. Blood and Rodstitis (1989) stated that the presence of *H. contortus* in the abomasum seems to interfere with digestibility of calcium. The increase in potassium level of infected lambs may be due to the fact that when the epithelia are damaged and their permeability increases the intracellular potassium may leak and defuse into the plasma, thereby increasing its contents. The increase in the phosphorus level reported here appears to be a reciprocal relation between calcium and phosphorus levels. Any increase in organic phosphorus is associated with the decrease in serum calcium (Tietz, 1982). The changes in sodium level reported here did not show any consistent pattern. The hypoproteineamia may also be attributed to the increased permeability of the abomasal mucosa leading to leakage of plasma. These minerals had progressive changes after treatment indicating an improvement of the animal's condition due to the treatment and decrease in the number of worm burden.

In this study, treatment with *B. aegyptiaca* and *A. herba alba* either before or after infection as well as albendazole did not show statistically significant effect on live weight gain of the animals. Nonetheless, no major conclusions on the long term effects of the preparation could be drawn for live weight gain, due to the short period of observation. This result implying that the differences in worm load had little effect on weight gain (Khan *et al*., 1988). Similar result was observed by Githiori *et al*. (2004) who observed no improvement in body weight gain of the treated animals. Also Equale *et al*. (2007) and Grade *et al*. (2008) didn't find any change in body weight in the treated lambs.

In the current study the abomasi contained dark red coloured fluid due to the presence of blood resulting from haemorrhage of the abomasal mucosa. Similar findings were reported following infection of lambs with *H. contortus* (Hunter and Mackenzie, 1982; Alzubaidy *et al*., 1987). The histopathological changes in the sections of the abomasi were generally in line with those of Malczewski (1970) and Salamn and Ducan (1984). They included mucosal and submucosal hemorrhages and cellular infiltration of the mucosa and submucosa with inflammatory cells. These findings were obvious in the untreated control group, followed by the groups treated before infection then by the group treated after infection. These differences may be due to differences in the number of adult worms found in the

abomasi resulting from different groups. The injury of the abomasal mucosa is related directly to the migration of larvae into the pits of gastric glands and also to the physical injury caused to the mucosa by the attachment of adult and pre adult stages of *H. contortus* (Blood and Rodostitis, 1989).

***In vitro* discussion**

The main advantage of using *in vitro* assay to study the anti-parasitic properties of the plants and plant extracts is the low costing and rapid turnover, which allow large number of plants to be screened. An additional advantage is the fact that these *in vitro* testing experiments measure directly the effect of anthelmintic activities based on the processes of development and motility of parasites without interference of any internal physiological functions of the host on pharmacodynamic and pharmacokinetic nature of the drug (Assis *et al*., 2003; Githiori *et al*., 2006; AlShaibani *et al*., 2009).

It is evident from this study that, the aqueous and methanolic extracts of *A.herba alba* and *B. aegyptiaca* exhibited significant anthelmintic activity against adult *H. contortus*. As recorded in the present study, anthelmintic effect of some plants had also been reported earlier against *H. contortus* (Igbal *et al*., 2004, 2005 and 2006; Jabbar *et al*., 2007; Eguale *et al*., 2007; Bachaya *et al*., 2009; Marie-Magdeleine *et al*., 2009 and 2010).

In the current study, *in vitro* results indicated that both aqueous and methanolic extracts of *A. herba alba* and *B. aegyptiaca* exhibited dose concentration- and time dependent anthemlntic effects by causing mortality of the worms. Similar results were obtained by Lateef *et al*. (2006) and Jabbar *et al*. (2007). However, in the absence of dose dependent factor the *in vitro* activity was also reported for other plants investigated earlier by Hounzangbe *et al*. (2005) and Eguale *et al*. (2007).

Significant anthelmintic effect of both extracts of *A. herba alba* on adult *H. contortus* was observed with greater activity in using methanolic extract than aqueous extract, these results confirmed the findings of earlier studies. For example, Igbal *et al*. (2004) found that, the alcoholic extract of *Artemisia brevifolia* was more effective in causing mortality of *H. contortus* as compared with the aqueous extract. Similarly the hydro-alcoholic extract of *Cariandrum sativum* and *Hedera helix* also showed better anthelmntic activity against *H. conortus* as compared with the aqueous extract (Eguale *et al*., 2007a, b).

Transcuticular diffusion is a common means of entry into helminth parasites for non nutrient and non electrolyte substances in nematodes (Alvarez *et al*., 2007). It has also been shown that this route is predominant for the uptake of major broad spectrum anthelminthics, by different nematodes, cestodes and trematode parasites as opposed to oral ingestion

(Geary *et al*., 1999). The possible explanation for the better anthelmintic activity of methanolic extract as compared with the aqueous extract could be due to a maximum concentration of alcohol soluble as an active anthelmintic principle in *A. herba alba*. Furthermore, the greater anthelmintic activity of the methanolic extracts in the current study could be due to easier and rapid transcuticular absorption of the ethanolic extract into the body of the worms owing to the lipid soluble nature of the ethanolic extracts (Eguale *et al*., 2007; Tariq *et al*., 2009). The anthelmintic activity of *A. herba alba* may be attributable to their Santonin content. Santonin is well known for its anthelmintic properties against hook worms (Jones, 1968).

In the present study, both types of extracts of *B. aegyptiaca* showed anthelmintic activity against *H. contortus*. Similar result was obtained by Ibrahim (1992) who reported that, one or more constituents mainly the saponins of *B. aegyptiaca* may be responsible for the anthelmintic activity. It is evident from the result of this study that, the aqueous extracts of *B. aegyptiaca* had higher activity as compared with the methanolic extracts, which may be considered as an indication of the presence of the saponins the water soluble which is active anthelmintic principle in *B. aegyptiaca*. Similar results were recorded by Igbal *et al*. (2005) who reported higher *in*

vitro anthelmintic efficacy of aqueous extract of *Calotropis procera* as compared with methanolic extract.

The results of this study showed that, the aqueous and methanolic extracts of *A. herba alba* and *B. aegyptiaca* as well as albendazole had no effect on the activity of third larval stage of *H. contortus*. This may be due to the strong survival ability of the third larval stage of *H. contortus* in different conditions because of being protected with the two cuticular sheaths (Hansen and Perry, 1994). The sheath of the L_3 inhibits or slows down the absorption of anthelmintics (Chappell, 1988).

In this study the results reported here showed the usefulness of *in vitro* studies to demonstrate the damage induced by the medicinal plants in *H. contortus* using transmission electron microscope. The effects of *A. herba alba* and *B. aegyptiaca* on adult *H. contortus* were not previously examined by ultrastructural studies.

A muscular hypertrophy was observed after treatment with *A. herb alba* which altered the tegumental muscle and mainly had paralysis effects. Sangester *et al*. (1991) have shown that, the levamisole induces a longitudinal contraction in some muscle fibers. Also similar result was reported by Beugnet *et al*. (1996). The microvilli of the intestines following treatment with *B. aegyptiaca* were spaced, some were lost, and others were irregular and were reduced in size. The reticulum was convoluted in the

form of concentric formation (like a finger print). On the other hand the intestines of *H. contortus* treated with *A. herba alba* showed large autophagic vacuoles. Alteration of microvilli, and the convoluted appearance of the reticulum were consistent with depolymerization of microtubules (lacey, 1990; Lubega and Prichard, 1991). Large autophagic vacuoles in the intestinal cells and concentric formations like finger prints in the rough endoplasmic reticulum were also observed by Beugnet *et al*. (1996). Also increased autophagic activity has been associated with a state of starvation or stress in the worms (Rothwell and Sangester, 1996). The worms treated with albendazole showed damaged mitochondrial wall and large numbers of autophagic vacuoles. Extensive studies of Aukstikalniene *et al*. (2000, 2005) had demonstrated the intestinal epithelial cells of *Toxocara canis* filled with vacuoles and granules, and associated degeneration of microvilli upon treatment with albemdazole and levamisole. Anthelmintics gave rise to lesions in relation to their known mode of action. Benzimidazoles inhibit the polymerization of microtubules (Lacey, 1990) and seem also to induce mitochondrial damage. McCradan and Sillwell (1991) showed that benzimedazole could have direct effects on uncouple oxidative phosphorylation. Elongated and swelling mitochondria are classical lesions of this type.

In the present work we found that both *A. herba alba* and *B. aegyptiaca* caused severe distortion with disorganization of the vetelline glands which showed numerous lipid droplets and vaculations.

Generally this transmission electron microscopic study following treatments with the two medicinal plants indicated consistent early changes in mitochondria; these changes were seen in all organs of the worms. In general the mitochondria became elongated, swollen, misshapen and disrupted. It is possible that these changes are associated with direct mitochondrial damage leading to a life threatening and decline in energy generation. These treatments are believed to act via inhibition of glycolosis (the main energy producing process). All these changes in mitochondria, the piled up of lipid deposits and the presence of autophagic vacuoles would be compatible with metabolic disruption of these tissues (Meaney *et al*., 2004).

Based on the results of the present study it can be concluded that, *A. herba alba* and *B. aegyptiaca* showed significant *in vitro* and *in vivo* anthelmintic activity at concentrations and doses tested against *H. contortus* as determined by worm motility inhibition of *H. contortus* and reduction in worm burden and faecal egg counts of *H. contortus* of sheep. These findings suggested that *A. herba alba* and *B. aegyptiaca* could form an alternative to commercially available synthetic anthelmintics; however

further investigations at different doses and concentrations against different parasitic stages and species are required to determine the true potentials of these plants (*A. herba alba* and *B. aegyptiaca*) as anthelmintics in the control strategies against gastrointestinal nematodes of sheep.

REFERENCES

Abakar, A. D. (1996). Coccidia and coccidiosis of sheep in Sudan, Ph.D. Thesis, University of Khartoum, Sudan.

Abbott, E. M.; Parkins, J. J. and Holmes, P. H. (1986). Effect of dietary protein on the pathogenesis of acute ovine haemonchosis. Veterinary Parasitology. **20**: 275-289.

Abu-Elfutuh, I. M. (1983). *Balanites aegyptiaca* and unutilized raw material potential ready of agro-industrial exploitation, Untied Nation Industrial Development Organization. Vienna, Austria, Report **10**.

Adam, S. E. I. (1978). Toxicity of indigenous plants and agricultural chemicals in farm animals. Clinical toxicology. **13**: 269-289.

Adams, D. B. (1983). Observation on the self-cure reaction and other forms of immunological responsiveness against *Haemonchus contortus* in sheep. International Journal for Parasitology. **13**: 571-578.

Adam, N. A. M. (2006). Effect of *piper Abyssinica* and *Jatropha curcas* against experimental *Haemochus contortus* infection in desert goats. M.V.Sc. Thesis, University of Khartoum, Sudan.

Ademola, I. O., Fagbemi, B. O., Idowu, S. O. (2004). Evaluation of the anthelmintic activity of *Khaya senegalensis* extract against gastrointestinal nematodes of sheep: in vitro and in vivo studies. Veterinary Parasitology. **122**: 151–164.

Ademola, I. O., Fagbemi, B. O., Idowu, S. O. (2007). Anthelmintic activity of Spigelia extract against gastrointestinal nematodes of sheep. Parasitol Res. **101**:63-69.

Ahmed, M. and Ansari, J. A. (1989). Effect of haemonchosis on haematology and non-specific phosphomonoestrase activities in sheep and goats. Helminthologia. **26**: 295-305.

Ahmed, A.; Anuar-ul Huassan, C.; Anuar-ul –Hag, A. and Majeed, M. A. (1990). Serum proteinogram of lambs in experimentally induced *Haemonchus contortus* infection. Veterinarsk. Archiv. **60**: 195-200.

Akhtar, M. S. (1984). Chemotherapy of ascariasis in local livestack. Pakistan Veterinary Journal. **4**: 75-80.

Akhtar, M. S. and Riffat, S. (1984). Efficacy of *Melia azedarach*, Linn. (Bahain) and morantel against naturally acquired gastrointestinal nematodes in goats. Pakistan Veterinary Journal. **4:** 176 - 179.

Akhtar, M. S. and Riffat, S. (1985). Evaluation of *Melia azedarach* Linn. fruit (Bahain) against *Ascaridia galli* infections in chickens. Pakistan Veterinary Journal. **5:** 34 - 37.

Akhtar, M.S., Javed, I. (1985). Comparative efficacy of *Fumaria parviflora* and morantel tartrate against gastrointestinal nematode infection in sheep. Pak. J. Pharmacol. **2**: 31–35.

Akhtar, M. S. and Riffat, S. (1987). Evaluation of anticestodal activity of *Lagenaria siceraria* (Kaddoo) seeds in sheep. Park Vet. J. **7**: 139-141.

Akhtar, M .S. and Javed, I. (1991). Efficacy of *Nigella sativa* linn. Seeds against *Moniezia* infection in sheep. Indian Vet. J. **68**: 726-729.

Aktrar, M. S. and Aslam, M. (1997). Anticostodal principles of *Nigella sativa*, Linn, Kolonji seeds. Pakistan J. PHarma. **14**: 7-14.

Akhtar, M. S.; Iqbal, Z.; Khan, M. N. and Lateef, M. (2000). Anthelmintic activity of medicinal plants with particular reference to their use in animals in the Indo-Pakistan subcontinent. Small Ruminant Research. **38:** 99 - 107.

Alawa, C. B. I.; Adamu, A. M.; Gefu, J. O.; Ajanusi, O. J.; Abdu, P. A.; Chiezey, N. P.; Alawa, J. N. and Bowman, D. D. (2003). *In vitro* screening of two Nigerian medicinal plants (*Vernonia amygdalina* and *Annona senegalensis*) for anthelmintic activity. Veterinary Parasitology **113**: 73 - 81.

Albers, G. A. A.; Gray, G. D.; Piper. L. R.; Barker, J. S. F.; Le-Jamber, L. F.; Barger, I. A. (1987). The genetic of resistance and resilience to *Haemonchus contortus* infection in young merino sheep. International Journal for Parasitology. **17**: 7, 1355-1363.

Albers, G. A. A.; Gray, G. D.; Le-Jambre, L. F.; Barger, I. A. and Barker, J. S. F. (1990). The effect of *Haemonchus contortus* infection on haematological parameters in young Merino sheep and its significance for productivity. Animal Production. **50**: 1, 99-109.

Allonby, E. W. and Dargie, J. D. (1973). Ovine haemonchosis in: Helminth Diseases of cattle, sheep and horses in Europe . G. M. Urquhart and J. Armour (editor). Glasgow Robert Maclehose Co. Ltd. The university press. Pp 59-81.

Allonby, E. W. and Urquhart, G. M. (1973). Self-cure of *Haemonchus contortus* under field condition. Parasitology. **66**:43-53.

Allonby, E. W. and Urquhart, G. M. (1975). The epidemiology and pathogenic significance of haemonchosis in a merino flock in East Africa. Veterinary Parasitology. **1**: 129 - 143.

Almazan, C., Avila, G., Quiroz, H., Ibarra, F., Ochoa, P., (2001). Effect of parasite burden on the detection of *Fasciola hepatica* antigens in sera and feces of experimentally infected sheep. Veterinary Parasitology. **97**: 101–112.

Al-Qarawi, A. A., Mahmoud, O. M., Haroun, E. M and Adam,S .E. L. (2001). A preliminary study of the anthelmintic activity of *Calotropis proceralatex* against *Haemonchus contortus* infection in Najdi sheep. Veterinary Research Communications.**25**: 61-70.

Al-Quaisy, H. H. K., Al-Zubaidy, A. J.; Altaif, K. I.; and Makkawi, T. A. (1987). Pathogenecity of haemochosis in sheep and goats in Irag, clinical, Parasitology and haematological findings. Veterinary Parasitology. **24**: 212-228.

Al-Shaibani, I. R. M, Phulam, M. S, Arijo, A , Qureshi, T. A, Shiekh, M. (2009). Anthelmintic activity of *Adhatoda vasica* against gastrointestinal nematodes of sheep. Pakistan journal of nematology. **27** (2): 255-268.

Al-Shamaony L, al-Khazraji S. M, Twaij H. A. (1994). Hypoglycaemic effect of *Artemisia herba alba*. II. Effect of a valuable extract on some blood parameters in diabetic animals**.** J Ethnopharmacol. **43**(3):167-71.

Alvarez, L. I., Lourdes Mottier, M. Lanusse, C. E. (2007). Drug transfer into target helminth parasites. Trends in Parasitology. **23** (3): 97-104.

Al-Zubaidy, A. J.; Altaif, K. I.; AlQuaisy, H. H. K. and Makkawi, T. A. (1987). Gross pathology and histology of haemonchosis in sheep and goats in Iraq. Veterinary Parasitology. **23**: 249-256.

Anderson, N. (1972). Trichostrongylid infections of sheep in a winter rainfall region. Epizootic studies in the western district of Victoria. *Aust. J. Agric. Res.* **24**: 499 - 611.

Andrews, F. W. (1952). The Flowering Plants of the Sudan, Vol. **II,** p. 486.

Anon, (1977). Manual of Veterinary Parasitological technique. Ministry of Agriculture, fisheries and food. Technical Bulletin No. 18, 2nd edition HMSO, London, pp. 131.

AOAD, (2001). Arab Organization for Agriculture Development.Year Statistical book, volume **20**.

Archibald, R. G., 1933. The use of the fruit of the *Balanites aegyptiaca* in the control of schistosomiasis in Sudan. Tropical Medicine and Hygiene. **27**: 207–210.

Armour, J.; Jenning, F. W. and Urquhart, G. M. (1969a). Inhibition of *Ostertagia ostertagi* at the early fourth larval stage. I: The seasonal incidence. Research in Veterinary Science. **10**: 232-237.

Armour, J.; Jenning, F. W. and Urquhart, G. M. (1969b). Inhibition of *Ostertagia ostertagi* at the early fourth larval stage. II:The influence of

environment on host or parasite. Research in Veterinary Science. **10**: 238-244.

Armour, J. and Bruce, R. G. (1974). The inhibition of development of *Ostertagia ostertagi*, adiapause phenomenon in a nematode. Parasitology. 69: 161-174.

Armour, J. (1978). The effect of weather factors on the inhibition of larval development in parasitic nematodes. In: weather and parasitic nematodes. In: weather and parasitic Animal Disease. Ed, T. E. Gibson, Technical note No. 159. Geneva: World Metrological Organization.

Artho, R.; Schnyder, M.; Kohler, L.; Torgerson, P. R. and Hertzberg, H. (2007). Ivermectin-resistance in gastrointestinal nematodes of Boer goats and Dorper sheep in Switzerland. Vet Parasitol. **144**(1-2): 68 - 73.

Asha, M. K.; Prashanth, D.; Murali, B.; Padmaja, R. and Amit, A. (2001). Anthelmintic activity of essential oil of *Ocimum sanctum* and eugenol. Fitoterapia. **72**: 669 - 670.

Assis, L. M.; Bevilaqua, C. M. L.; Morais, S. M.; Vieira, L. S.; Costa, C. T. C. and Souza, J. A. L. (2003). Ovicidal and larvicidal activity *in vitro* of *Spigelia anthelmia* Linn. Extracts on *Haemonchus contortus*. Veterinary Parasitology. **117**: 43 - 49.

Aukstikalniene, R.; Kublickiene, O.; Vysniauskas, A.; Zilinskiene, R. (2000). Histological investigations of *Toxocara canis* tissues and their micro morphological changes under the action of albendazole *in vivo*. Acta zool lithuanica. **10**: 74-84.

Aukstikalniene, R.; Kublickiene, O.; Vysniauskas, A. (2005). The influence of different anthelmintics on the intetinal epithelial tissue of *Toxocara canis* (nematoda). Vet zootech. **29**: 9-16.

Averkin, E. A.; Beard fried, J. H.; Killian, J. G. and Schilts, R. A. (1975). Methyl 5 (6) phenylsulfinyl 2-benzimidezole olecarbamate a new potent anthelmintic. Journal of Medical Chemistry. **18**: 1164-1166.

Bachaya, H., Igbal, Z., Khan, M. N., Sindhu, Z. D. and Jabbar (2009). Anthelmintic activity of *Ziziphus nummularia* (bark) and *Acacia nilotica* (fruit) against trichostrongylid nematodes of sheep. Journal of Ethnopharmacology. **123**: 325-329.

Baker, R. L. (1998). A review of genetic resistance to gastrointestinal nematode parasites in sheep and goats in the tropics and evidence for resistance in some sheep and goat breeds in sub-humid coastal Kenya. Animal Genetic Resources Information. **24:** 13 - 30.

Baker, R. L.; Mwamachi, D. M.; Audho, J. O.; Aduda, E. O.; Thorpe, W. (1999). Genetic resistance to gastrointestinal nematode parasites in Red Maasai, Dorper and Red Maasai x Dorper ewes in the sub humid tropics.

Baker, R. L.; Nagda, S.; Rodriguez Zas, S. L.; Southey, B. R.; Audho, J. O.; Aduda, E. O. and Thorpe, W. (2003). Resistance and resilience to gastro-intestinal nematode parasites and relationships with productivity of Red Maasai, Dorper and Red Maasai & Dorper crossbred lambs in the sub-humid tropics. Animal Science .**76**: 119 - 136.

Bally,P. R. O. (1937). Native medicinal and poisonous plants of East Africa. Kew Bull.**1**:10-25.

Bang, K. S.; Familton, A. S. and Sykes, A. R. (1990). Effect of copper oxide wire particle treatment on establishment of major gastrointestinal nematodes in lambs. Research in Veterinary Science. **49**: 132 - 137.

Banks D. J. D. (1988). Potential parasitic problems in the S. W. Pacific Region. Proceedings of the Papua New Guinea Society of Animal Production, Vol. **I**, pp. 89-94. Inaugural Conference, Lae, Morobe Provence.

Banks, D. J. D.; Singh, R.; Barger, I. A.; Pratap, B. and Le Jambre, L. F. (1990). Development and survival of infective larvae of *Haemonchus contortus* and *Trichostrongylus colubriformis* on pasture in a tropical environment. International Journal for Parasitology. **20**: 155 - 160.

Barger I. A. and Southcott W. H. (1978). Parasitism and production in weaner sheep grazing alternately with cattle. Australian Journal of Experimental Agriculture and Animal Husbandry. **18**: 340-346.

Barger, I. A.; Lewis, R. J. and Brown, G. F. (1984). Survival of infective larvae of nematode parasites of cattle during drought. Vet Parasitol.**14**: 143 - 152.

Barger, I. A.; Siale, K.; Banks, D. J. D. and Le Jambre, L. F. (1994). Rotational grazing for control of gastrointestinal nematodes of goats in a wet tropical environment. Vet Parasitol.**53**: 109 – 116.

Barger, I. A. (1999). The role of epidemiological knowledge and grazing management for helminth control in small ruminants. International Journal for Parasitology. **29**: 41-47.

Barnes, E. H.; Dobson, R. J. and Barger, I. A. (1995). Worm control and anthelmintic resistance: adventures with a model. Parasitology Today. **11**: 56 - 63.

Bartley, d., Jackson, E.E., Jckson,K., coop,R.L., M itchell, B.B., Sales, J. and Jackson, F. (2003). Asurvey of anthelmintic resistant nematode parasites in scottish sheep Flocks. Vet. Parasitol. **117**:61-71.

Bashir, A.K., Elhadi, M.M., Elkheir, Y.M., (1984a). Investigation of molluscicidal activity of certain Sudanese medicinal plants used in folk-medicine, Part IV. Planta Medica **74**:111–116.

Bashir, A.K, Ahmed, G.H, Suliman, S.M, ElKheir, Y.M. Molluscicidal and other biological activities of Balanites aegyptiaca. (1984b). the first Arab conference on medicinal plants. Egypt, Cairo.

Bedevian, A. K. (1936). Illustrated polyglottic Dictionary of plants names in Latin, Arabic, American, English, French, German, Italian and Turkish languages. Argus and Papazian presses, Cairo.

Benitez-Usher C, Armour J, Duncan J. L, Urquhart G. M, Gettinby G. A. (1977). Study of some factors influencing the immunization of sheep against Haemonchus contortus using attenuated larvae. Vet Parasitol. 3: 327-342.

Berger, J. (1975).The resistance of a field strain of Haemonchus contortus to five Benzimidazole anthelmintics in current use. Journal of the South Africa Veterinary Association. 6:112-15

Beugnet. F. Kerboeuf. D. , Nicolle. J.C. Soubieux. D. (1996). Use of free living stages to study the effects of thiabendazole, levamisole, pyrantel and ivermectin on the fine structure of Haemonchus contortus and Heligmosomoides polygyrus. Veterinary Parasitology. 63: 83-94.

Bizimenyera, E. S., J. B. Githiori, J. N. Eloff and G. E. Swan. (2006). In vitro activity of Peltophorum africanum Sond (Fabaceae) extracts on the egg hatching and larval development of the parasitic nematode Trichostrongylus colubriformis. Vet. Parasitol. 142: 336–343

Bizimenyera, E. S. Meyer, S. Naidoo, V. Eloff, J. N. and Swan, G. E. (2008) Efficacy of Peltophorum africanum Sond. (Fabaceae) Extracts on Haemonchus contortus and Trichostrongylus colubriformis in Sheep. Journal of Animal and Veterinary Advances. 7: (4) 364-371

Blood, D. C. and Radostitis, O. M. (1989). Veterinary Medicine, A text book of the Disease of cattle, sheep, pigs, Goats and Horses. 7th edition. Bailliere Tindall, Oxford University Press. 1502pp.

Boisvenue, R.J., Stiff, M.I., Tonkinson, L.V., Cox, G.N. (1991). Protective studies in sheep immunised with cuticular collagen proteins and peptides of Haemonchus contortus. Parasite Immunol. 13 (3), 227–240.

Boriky, D., Berrada, M., Talbi, M., Keravis, G. and Rouessac, F. (1996). Eudesmanolides from Artemisia herba–alba .Photochemistry. 43: 309-311.

Boughton, L. B. (1940). Phenothiazine for stomach worms of sheep and goats, southwest sheep. Goat Raiser. 11(3): 32-33.

Bowman, D. D., Lynn, R. C. & Georgi, J. R. (2003). Georgi's parasitology for veterinarians. 8th edition. W. B. Saunders, 408 pp. ISBN 0721692834.

Bradley, R. E.; Radhakishan, C. V.; Patial-Kulkarni, V. G. and Loggins, P. E. (1973). Responses in Florida Native and Rambouillet lambs exposed to

one and tow oral doses of Haemonchus contortus. American Journal of Veterinary Research. 34: 729-735.

Broun, A. F., Massey, R. E, (1929). Flora of the Sudan. Thomas Marby and Co, London.

Buijs, J. and Buitenberg, E. J. (1987). Immunopathology of intestinal nematode infection. Bailliere's Clinical and Tropical Medicine and Communicable Diseases. 2: 535-551.

Caroline Komen, Frederick, M. Wanjala, Paul C. Kiprono. (2005). Efficacy of Jasminum abyssinicum treatment against Haemonchus contortus in sheep. Afr. J. Trad. CAM . 2 (3): 264 – 268.

Chappell, L. H., (1988). The interactions between drugs and the parasite surface. Parasitology. 96.

Charles I. Muleke, Ruofeng Yan, Yanming Sun, Guangwei Zhao, Lixin Xu, Xiangrui Li. (2007). Vaccination of goats against Haemonchus contortus with a recombinant cysteine protease. Small Ruminant Research. 73: 95–102

Charleston, W. A. G. (1965). Pathogenesis of experimental heamochosis in sheep, with special reference to the development of resistance. Journal of Comparative Pathology and Therapeutics. 75: 55-67.

Chartier, C.; Etter, E.; Hoste, H.; Pors, I.; Koch, C. and Dellac, B. (2000). Efficacy of copper oxide needles for the control of nematode parasites in dairy goats. Veterinary Research Communications. 24: 389 - 399.

Chartier, C.; Pors, I.; Hubert, J.; Rocheteau, D.; Benoit, C. and Bernard, N. (1998). Prevalence of anthelmintic resistant nematodes in sheep and goats in Western France. Small Rumin. Res. 29: 33 – 41.

Chartier, C.; Soubirac, F.; Pors, I.; Silvestre, A.; Hubert, J.; Couquet, and Cabaret, J. (2001). Prevalence of anthelmintic resistance in gastrointestinal nematodes of dairy goats under extensive management conditions in southwestern France. J. Helminthol. 75: 325–330.

Chiejina, S. N.; Fake, B. B. and Eze, P. L. (1989). Development and survival of free living stages of gastrointestinal nematodes of sheep and goats on pasture in Nigerian derived savanna. Veterinary Research Communication. 13: 103-112.

Chitwood, M. B. (1969). Systematics and biology of some parasitic nematodes in: Chemical zoology. M. Florkin and B. T. Scheer (Editors), volume III. PP. 223-244.

Choi, M. H., Park, I. C., Li, C., Hong, S.T. (2003). Excretory-secretory antigen is better than crude antigen for the serodiagnosis of clonorchiasis by ELISA. Korean Journal Parasitology. 41: 5–9.

Chopra, R. N., S. L. Nayyar and I. C. Chopra. (1956). Glossary of Indian Medicinal Plants, p. 160. Council of Scientific and Industrial Research, New Delhi, India

Clark, C. H.; Kiesel, G. K. and Goby, C. H. (1962). Measurement of blood loss caused by Haemonchus contortus infection in sheep. American Journal of Veterinary Research. 23: 977-980.

Coles, G. C.; Jackson, F.; Pomroy, W. E.; Prichard, R. K; von Samson-Himmelstjerna, G.; Silvestre, A.; Taylor, M. A. and Vercruysse , J. (2006). The detection of anthelmintic resistance in nematodes of veterinary importance. Veterinary Parasitology.136: 167–185.

Colglazier, L. L.; Kates, K. C. and Enzie, F. D. (1972). Activity of cambendazole and morantel tartarate against tow species of trichostrogylus and tow thiabendazole resistant isolates of Haemonchus contorus in sheep. Proceeding of the Helminthological Society of Washington. 39: 28-32.

Costa, C. A. F.; Vieira, L. da S.; Berne, M. E. A.; Silva, M.U.D.; Guidoni, A. L.; Figueiredo, E. A. P. (2000). Variability of resistance in goats infected with Haemonchus contortus in Brazil. Veterinary Parasitology. 88: 153-158.

Costa, C.T.C., Bevilaqua, C.M.L., Maciel, M.V., Camurc¸a-Vasconcelos, A.L.F., Morais, S.M., Monteiro, M.V.B., Farias, V.M., Silva, M.V., Souza, M. M. C.(2006). Anthelmintic activity of Azadirachta indica A Juss against sheep gastrointestinal nematodes. Vet. Parasitol. 137: 306–310.

Crofton, H. D. (1963). Nematode parasite population in sheep and on pasture. Technical communication No. 35, Commonwealth Bureau of Helminthology, p.104.

Cushny, A. R. (1908). Nutmeg poisoning. Proc Royal Soc. 3:39-44.

Cvetkovic, L. J.; Cuperlovic, K.; Movsesijan, M.; Lalic, R. (1978). Genetic resistance of sheep to infection with Haemonchus contortus. Acta Parasitological Yugoslavia. 9: 2, 75-79.

Dargi, J. D. (1975). Application of radio-isotope techniques to the study of red cell and plasma protein metabolism in helminth diseases of sheep. Symposium of the Britsh Society of Parasitology. 31: 1-26.

David, W. Halton (2004). Microscopy and the helminth parasite. Micron. 35: 361–390.

Dhar, R. N., Garg, L. C. and Pathak, R. D. (1965). Anthelmintic activity of Carica papya seeds. Indian J. Pharm. 27: 335-336.

Didem, T., Mustafa, A., Gulsa, H.A., Mustafa, K. and Ayhan, T. (2006). Hypoglycaemia effect of Artemisia herbaba alba in experimental hyperglycaemic rats. Bull. Vet. Inst. Pulary. 50.:235-238.

Dineen, J. K.; Donald A. D.; Wagland, B. M. and offner, J. (1965). The dynamic of the host-parasite relationship. I The response of sheep to primary infection with Haemonchus contortus. Parasitology. 55: 515-525.

Donald A. D., Morley F. H. W., Axelsen A., Donnelly J. B. & Wailer P. J. (1987). Integration of grazing management and anthelmintic treatment for the control of nematode infections in young sheep. In: Temperate Pastures, Their Production, Use and Management (Edited by WheelerL., Pearson C. J. & Robards G. E.), pp. 5677569. Australian Wool Corporation/CSIRO, Melbourne.

Dorny, P., Claerebout, E., Vercruysse, J., Sani, R., Jalila, A., (1994). Anthelmintic resistance in goats in peninsular Malaysia. Vet. Parasitol.55: 327–342.

Drudge, J. H.; Leland, S. E. J. and Wyant, Z. M. (1957). Strain variation in the response of sheep nematodes to the action of phenothiazine. Studies of mixed infections in experimental animals. American Journal of Veterinary Research. 18: 133-141.

Drury, R. A. B and Walligton, E. A. (1967). Carleton,s Histological Techniques, 4 edition, London, Oxford University Press. 432pp.

Dubey, M.P. and Gupta, I. (1968). Studies on the anthemintic activity of A. languium (amarikii root bark. Indian J. phys. Pharmal. 12: 25-31.

Duncan, J. L., Smith, W. D. and Dargie, J. D. (1978). Possible relationship of levels of mucosal IgA and serum IgG to immune unresponsiveness of lambs to Haemonchus contortus. Veterinary Parasitology. 4: 21.

Dunn, A. M. (1978). Veterinary Helminthology. William Heinemann Medical Books LTd. London second edition. PP 172-195.

Eguale, T., Tilahun, G., Debella, A., Feleke, A., Makonnen, E., (2007a). In vitro and in vivo anthelmintic activity of crude extracts of Coriandrum sativum against Haemonchus contortus. J. Ethnopharmacol. 110:428–433.

Eguale, T., Tilahun, G., Debella, A., Feleke, A., Makonnen, E., (2007b). Haemonchus contortus: In vitro and in vivo anthelmintic activity of aqueous and hydro-alcoholic extracts of Hedera helix. Exp. Parasitol. 116: 340–345.

Eguale, T.; Chaka, H. and Gizaw, D. (2009). Efficacy of albendazole against nematode parasites isolated from a goat farm in Ethiopia: relationship between dose and efficacy in goats. Trop Anim Health Prod.

El Bihari, S., Kawasmeh, Z.A., Ashour, N.A., and El Naiem, A.U. (1984). Experimental infection of sheep by the camel stomach worm Haemonchus Longstips. Veterinary Parasitology. 15: 257-261.

El Hassan, R. O. (2002). Experimental infection of three Sudanese sheep breeds to Haemonchus contortus. Thesis. university of Khartoum, Sudan.

ELSheikh, S. H. (1994). Biological and chemical studies on molluscicidal and schistosomocidal components with special reference to Balanites aegyptiaca (L.) Del. Constituent, Ph.D. Thesis, University of Khartoum, Sudan.

El-Taieb, S. A. M.. (1995). Comparative efficacy of Mebendazole, Cheurbita maxima and Tapiney against, Raillietina tetragona infection in chickens. M.V.Sc. Thesis, University of Khartoum, Sudan.

Emery, D. L. and Wagland, B. M. (1991). Vaccines against gastrointestinal nematode parasites of ruminants. Parasitology Today. 7: 347-349.

Eskander, A. M. (1982). Pharmacological and Toxicological Effects of Balanites aegyptiaca on Laboratory Animals. M. Vet. Sci. Thesis, University of Khartoum, Sudan.

Eysker, M.; van Graafeiland, A. E. and Ploeger, H. W. (2006). Resistance of Teladorsagia circumcincta in goats to ivermectin in the Netherlands. 131(10): 358 – 361.

Fabiyi, J.P.(1987). Production losses and control of helminthes in ruminants of tropical regions. International Journal for parasitology.17:435-441.

Forsyth, B. A.; Scott, M. T. and Bainbridge, J. R. (1961). Phenothiazine. The effect of purity and dose on its anthlelmintic efficiency in sheep. Veterinary Record. 73: 67-75.

Gagoad, I. M. H and Eisa, A. M. (1968). Incidence and intensity of helminth parasites of sheep in the Sudan. Part 1. Parasites of the abomasum. Sudan Journal of Veterinary Science and Animal Husbandry. 9: 344-354.

Galal, M.; Bashir, A. K.; Salih, A. M. and Adam, S. E. (1991 a). Activity of water extracts of Albizzia anthelmintica and A. lebbek barks against experimental Hymenolepis diminuta infection in rats. Journal of Ethnopharmacology. 31: 333 - 337.

Galal, M.; Bashir, A. K.; Salih, A. M. and Adam, S. E. (1991 b). Efficacy of aqueous and butanolic fractions of Albizzia anthelmintica against experimental Hymenolepis diminuta infestation in rats. Veterinary and Human Toxicology. 33: 537 - 539.

Gamble, H. R. and Zajac, A. M. (1992). Resistance of St. Croix lambs to haemonchus contortus in experimentally and naturally acquired infections. Veterinary Parasitology. 41: 211-225.

Gamble, H.R., Rapic, D., Marinculic, A., Murrell, K.D., (1988). Evaluation of excretory-secretory antigens for the serodiagnosis of swine trichinellosis. Veterinary Parasitology. 30: 131–137.

Garag, S. C. and Jain, R. (1992). Biological activity of the essential oil of piper batle, L.J. Essential Oil Res. 4: 601-606.

Gatongi,. P. M; Prichard, K. R.; Ranjan, S.; Gathuma, M. J.; Munyua, K W.; Cheruiyot, H. and Scott, E. M. (1998). Hypobiosis of Haemonchus contortus in natural infections of sheep and goats in a semi-arid area of Kenya. Veterinary Parasitology. 77(1):49 –61.

Geary, T. G., Thompson, D. P., Klein, R. D. (1999). Mechanism-based screening: discovery of the next generation of anthelmintics depends upon more basic research. International Journal of Parasitology. 29: 105–12.

Gebrekiros Asegede. (1990). Studies on the Ecology of Helminth Parasites in Naturally Infected Indigenous Sheep in Awassa, Southern Ethiopia. PhD thesis, Center of Tropical Sciences and Parasitology, Giessen University Germany, Germany. 176 pp.

Ghulam Rasool; Zafar Tghal; Khan, N. M. and Hayat, B. (1995). Haematological disturbances associated with haemonchsis in sheep. PakistanVeterinary Journal.15(4): 159-162.

Gibbs, H. C. (1986). Hypobiosis and periparturient rise in sheep. Vet. Clinics of North America: Food Animal Practice. 2(2).

Gibbson, M. L. (1979). Revision of the genus heamonchus, Cobb 1898 (Nematode: Trichostrongylidae). Systematic Parasitology. 1: 3- 24.

Gill, B. S. (1993). Anthelmintic resistance in India. The Veterinary Record 133: 603-604.

Gill, J. H.; lacey, E. (1998). Avermectin/milbemycin resistance in trichostrongyloid nematodes. International Journal forParasitology. 28(6): 863-77.

Githigia, S. M.; Thamsborg, S. M.; Larsen, M.; Kyvsgaard, N. C. and Nansen, P. (1997). The preventive effect of the fungus Duddingtonia flagrans on trichostrongyle infections of lambs on pasture. International Journal for Parasitology. 27: 931-939.

Githiori, J. B., Ho¨ glund, J., Waller, P. J., Baker, R. L. (2002). Anthelmintic activity of preparations derived from Myrsine Africana and Rapanea melanophloeos against the nematode parasite, Haemonchus contortus of sheep. J. Ethnopharmacol. 80: 187–191.

Githiori, J. B., Ho¨glund, J., Waller, P. J., Baker, R. L. (2003). The anthelmintic efficacy of the plant, Albizia anthelmintica, against the nematode parasites Haemonchus contortus of sheep and Heligmosomoides polygyrus of mice. Vet. Parasitol. 116: 23–34.

Githiori, J. B., glund, J. H. O., waller, P. J. And baker, R. L. (2004). Evaluation of anthelmintic properties of some plants used as livestock dewormers against Haemonchus contortus infections in sheep. Parasitology. 129: 245–253.

Githiori, J. B., Athanasiadou, S., Thamsborg, S. M. (2006). Use of plants in novel approaches in control of gastrointestinal helminthes in live stock with emphasis on small ruminants. Vet. Parasitol. 139: 308–320.

Gordon, H. McL. (1948). Epidemiology of parasitic diseases, with special reference to studies with nematode parasites of sheep. Australian Veterinary Journal. 24: 17-44.

Gradé, J. T.; Arble, B. L.; Weladji, R. B. and Van Damme, P. (2008). Anthelmintic efficacy and dose determination of Albizia anthelmintica against gastrointestinal nematodes in naturally infected Ugandan sheep. Vet. Parasitol. 157(3-4): 267 - 274.

Gray, G. D. (1987). Genetic resistance to haemonchosis in sheep. Parasitology Today. 3 (8): 253-255.

Gray, G. D. (1997). The use of genetically resistant sheep to control nematode parasitism. Veterinary Parasitology. 72: 345-366.

Gray, G. D.; Presson B. L.; Albers, G. A. A.; Le- Jambre, L. F.; Piper, L. R. and Barker, J. S. F. (1987). Comparison of within and between breed variations in resistance to haemonchosis in sheep. Merino Improvement programs in Australia. . Ed B. J. McGuirk. Melbourne, Australian Wool Corporation. PP 365-369.

Gronvold J., Wolstrup J., Larsen M. & Henriksen S. A. (1993). Nematode-trapping fungi against parasitic cattle nematodes. Parasitology Today. 9: 137-140.

Gruner, L., Gouix, J., Cabaret, J., Boulard, C., Cortet, J., Sauve, C., Molenat, G. and Calamel, M. (1992). Effect of genetic type lactation and management on helminth infection of ewes in an intensive grazing system on irrigated pasture. INt. J. Parasitol. 22: 919-925.

Hammond, J. A.; Fielding, D. and Bishop, S. C. (1997). Prospects for plant anthelmintics in tropical veterinary medicine. Veterinary Research Communications. 21: 213-228.

Hansen, J. and Perry, B. (1994). The epidemiology, diagnosis and control of helminth parasites of ruminants: A handbook. International Livestock Research Institute, Nairobi, 171 pp. ISBN 9290557031.

Harborne, J. B. (1984). Phytochemical methods. 2nd edition. Chapman and Hall.

Harder, A. (2002). Chemotherapeutic approaches to nematodes: current knowledge and outlook. Parasitology Research. 88: 272-277.

Harmon, F. A.; Williams, B. Z.; Zarlenga, S. D. and Michael B. Hildreth, B. M. (2007). Real-time PCR for quantifying Haemonchus contortus eggs and potential limiting factors. Parasitology Research.101(1): 71 – 76.

Hennessy, D.R., (1997). Modifying the formulation or delivery mechanism to increase the activity of anthelmintic compounds. Vet. Parasitol. 72: 367–390.

Herlich, H. (1956). A digestion method for post-mortum recovery of nematodes from ruminants. Proceedings of the Helminthological Society of Washington. 23: 102-103.

Hördegen, P.; Hertzberg, H.; Heilmann, J.; Langhans, W. and Maurer, V. (2003). The anthelmintic efficacy of five plant products against gastrointestinal trichostrongylids in artificially infected lambs. Veterinary Parasitology. 117: 51-60.

Hördegen, P.; Cabaret, J.; Hertzberg, H.; Langhans, W.; Mau. V. (2006). In vitro screening of six anthelmintic plant products against larval Haemonchus contortus with a modified methyl-thiazolyl-tetrazolium reduction assay. Journal of Ethnopharmacology. 108: 85–89.

Hotez, P., Haggerty, J., Hawdon, J., Milstone, L., Gamble, H. R., Schad, G., Richards, F., (1990). Metalloproteases of infective ancylostoma hookworm larvae and their possible functions in tissue invasion and ecdysis. Infection and Immunity. 58: 3883–3892.

Hounzangbe-Adote, M. S., Paolini, V., Fouraste, I., Moutairou, K., Hoste, H., (2005). In vitro effects of four tropical plants on three life-cycle stages of the parasitic nematode, Haemonchus contortus. Research in Veterinary Science. 78:155–160.

Howell, S. B.; Burke, J. M.; Miller, J. E.; Terrill, T. H.; Valencia, E.; Williams, M. J.; Williamson, L. H.; Zajac, A. M. and Kaplan, R. M. (2008). Prevalence of anthelmintic resistance on sheep and goat farms in the southeastern United States. J Am Vet Med Assoc. 233(12): 1913 – 1919.

Hunter, A. R. and Machenzie, G. (1982). The pathogenesis of a single challenge dose of Haemonchus contorus in lambs under six month of age. Journal of Helminthology. 56: 135-144.

Ibrahim, A. M., (1992). Anthelmintic activity of some Sudanese medicinal plants. Phytother. Res. 6, 155–157.

Ibrahim, M. A.; Nwude, E.; Ogunsusi, R. A. and Aliu, Y. O. (1984). Screening of West African plants for anthelmintic activity. ILCA Bulletin No. 17. International Livestock Centre for Africa, Addis Ababa, Ethiopia, pp. 19-23.

Idris, U. E., Adam, S. E., Tartour, G., (1982). The anthelmintic efficacy of Artemisia herba-alba against Haemonchus contortus infection in goats. Natl. Inst. Anim. Health Q. (Tokyo) 22: 138– 143.

Iqbal, Z.; Nadeem, Q. K.; Khan, M. N.; Akhtar, M. S. and Waraich, F. N. (2001). In vitro nthelmintic activity of Allium sativum, Zingiber officinale, Curcurbita mexicana and Ficus religiosa. International Journal of Agriculture and Biology. 3: 454-457.

Iqbal, Z., Lateef, M., Ashraf, M., Jabbar, A. (2004). Anthelmintic activity of Artemisia brevifolia in sheep. J. Ethnopharmacol. 93: 265–268.

Iqbal, Z. Jabbar, A., Akhtar, M. S., Muhammad, G., Lateef, M. (2005). Possible Role of Ethnoveterinary Medicine in Poverty Reduction in Pakistan: Use of Botanical Anthelmintics as an Example. Journal of Agriculture and Social Science. 2: 187-195.

Iqbal, Z., M. Lateef, A. Jabbar, M. S. Akhter and M. N. Khan, (2006a).Anthelmintic activity of Vernonia anthelmintica seeds against Trichostrongylid nematodes of sheep. Pharm. Biol. 44: 563–567.

Iqbal, Z., Lateef, M., Akhter, M.S., Gayur, M.N., Gilani, A.H. (2006b). In vivo anthelmintic activity of ginger against gastrointestinal nematodes of sheep. J. Ethnopharmacol. 106: 285–287.

Iqbal, Z., Lateef, M., Khan, M. N., Jabbar, A., Akhter, M. S., (2006b). Anthelmintic activity of Swertia chirata against gastrointestinal of sheep Fitoterapia. 77: 463–465.

Jabbar, A., Z. igbal, D. Kerboeuf. G. Muhammed , M. N. Khan and M. Afaq. (2006). Anthelmintic resistance the state of play revisited. Life Sci., 79:2413-2431.

Jabbar, A., Zaman, M. A., Iqbal, Z., Yaseen, M., Shamim, A. (2007). Anthelmintic activity of Chenopodium album (L) Caesalpinia crista (L.) against trichostrongylid nematodes of sheep. J. Ethnopharmacol.114: 86–91.

Jacob Yashphe, Ruth Segal, Aviva Breuer, Gabi Erdreich-Naftali Antibacterial activity of Artemisia herba-alba. (1979). Journal of Pharmaceutical Sciences. **68**(7): 924 - 925

Jain, N. C. (1986). Schalm's Veterinary Heamatology. 4th Edition. Philadelphia. :Lea and Febiger. 1221pp.

Jambre, L. F. LE.; Southcott, W. H. and Dosh, K. M. (1976). Resistance of selected type of Haemonchus contortus to Thiabendazole Monotrtrate and Levamisole. International Journal for Parasitology. 6: 212-222.

Jones, L.M. (1968). Veterinary pharmacology and therapeutics, 3rd edition, Iowa State University Press, Ames, Iowa, USA.

Jarrett W. F. H.; Jennings F. W.; McIntyre W. I. M.; Mulligan W. and Sharp N. C. C. (1959). Studies on immunity to Haemonchus contortus infection-vaccination of sheep using a single dose of X-irradiated larvae. American Journal of Veterinary Research. 20: 527-531.

Jarrett WgFH, Jennings FW. McIntyre WIM. Mulligan W, Sharp NCC. (1961). Studies on immunity to Haemonchus contortus infection of sheep double vaccination of sheep with irradiated larvae. Am J Vet Res. 20: 186 - 188.

Kabagambe, E.K.; Barras, S.R.; Li, Y.; Pena, M.T.; Smith, W.D.and Miller, J. E. (2000). Attempts to control haemonchosis in grazing ewes by vaccination with gut membrane proteins of the parasite. Veterinary Parasitology. 92: 1, 15-23.

Kahiya, C.; Mukaratirwa, S. and Thamsborg, S. M. (2003). Effects of Acacia nilotica and Acacia karoo diets on Haemonchus contortus infection in goats. Veterinary Parasitology. 115: 265-274.

Kailani, S. U. R., Akhtar, M. S. and Ashraf, M. (1995). Antifasciotic efficacy of indigenous plant drugs: Kalonji, Shahteran and Karanjwa in buffaloes. Pakistan J. Pharm. Sci. 8: 17-27.

Kasonia, K., Ansay, M. ,Basegere, N. ,Gustin, P.,Kaba, S. ,Katsongeri, M., Matamba, M., (1991). Note d'ethnopharmacologie veterinaire en cas de verminoses, diarrhoea, corprostase et meteorisme au Kivu et Kibali-ituri (Zaire). Tropicultura. 9: 169–172.

Kates, K. C. (1950). Survival on pasture of free-living stages of some common gastrointestinal nematodes of sheep. Proc. Helminthol. Soc., Washington.**17**: 39 - 50.

Ketzis, J. K., Taylor, A., Bowman, D. D., Brown, D. L., Warnick, L. D., Erb, H. N. (2002). Chenopodium ambrosioides and its essential oil as treatments for Haemonchus contortus and mixed adult-nematode infections in goats. Small Ruminant Res. 44: 193–200.

Khalid, H. S. (1992). Biological and phytochemical studies on Albizia anthelmintica. Ph.D. Thesis, University of Khartoum, Sudan.

Khalid, F. A., ABdalla, N. M., Mohomed, H. O, Toum, A. M., Magazoub, M. M. A., Ali, M. S. (2005). In vitro Assessment of Anti - Cutaneous Leishmaniasis Activity of Some Sudanese Plants. Acta Parasitologica Turcica. 29 (1): 3-6.

Khan, M. Q.; Hayat, S.; Ilyas, M.; Hssain, M. and Igbal, Z. (1988). Effect of haemonchosis on body weight and blood volumes in sheep. Pakistan Veterinary Journal. 8:62-67.

Khunkitti, W., fujimaki, Y. and Aoki, Y. (2000). In vitro antifilaricidal activityof extracts of the medicinal plant Cartiosperum helicacabum against Brugia phangi. Journal of Helminthology. 74:241-246.

Kirsch, R. and Duwel, D. (1975). Laboratory investigations in sheep with a new anthelmintic. Veterinary Record. 97: 28-31.

Kloosterman, A.; Parmentier, H. R. and Ploeger, H. W. (1992). Breeding cattle and sheep for resistance to gastrointestinal nematodes. Parasitology Today. 8: 330-335.

Knight, R. E.; Vegirs, H. H. and Glimp, H. A. (1973). Effects of breed and date of birth of lambs on gastrointestinal nematode infections. American Journal of Veterinary Research. 34: 323-327.

Knox, M. R. (2002). Effectiveness of copper oxide wire particles for Haemonchus contortus control in sheep. Australian Veterinary Journal. 80: 224-227.

Koko, W. S.; Galal, M. and Khalid, H. S. (2000). Fasciolicidal efficacy of Albizia anthelmintica and Balanites aegyptiaca compared with albendazole. Journal of Ethnopharmacology. 71: 247-252.

Koko, W.S., Abdallab, H.S., Galala, M., Khalida, H.S.(2005). Evaluation of oral therapy on Mansonial Schistosomiasis using single dose of Balanites aegyptiaca fruits and praziquantel. Fitoterapia. 76:30–34.

Kokwaro, J. O. (1993). Medicinal plants of East Africa. 2nd edition. Kenya Literature Bureau, Nairobi, 401 pp. ISBN 9966441905.

Lacey, E. (1990) Mode of action of Benzimidazoles. Parasitol Today. 6:112–115

Lal, J., Chandra, S., Prakash, V. R. and M. Sabir, (1976). In vitro anthelmintic action of some indigenous medicinal plants on Ascaridia galli worms. Indian J. Physiol. Pharmacol. 20: 64–8.

Larsen, M. (1999). Biological control of helminths. International Journal for Parasitology. 29: 139-146.

Larsen, M. (2000). Prospects for controlling animal parasitic nematodes by predacious micro fungi. Parasitology. 120: 121–131.

Lateef, M.; Iqbal, Z., Khan, M. N.; Akhtar, M. S. and Jabbar, A. (2003). Anthelmintic activity of Adhatoda vesica roots. International Journal of Agriculture and Biology. 5(1): 86-90.

Lateef, M.; Iqbal, Z.; Akhtar, M. S. and Jabbar, A. (2006). Preliminary screening of Trachyspermum ammi (L.) seed for anthelmintic activity in sheep. Trop Anim Health Prod. 38: 491-496.

Le-Jambre, L. F. (1978). Host genetic factors in helminth control In: The epidemiology and control of Gastrointestinal parasites of sheep in Australia (Edited by Donald A. D., Southcott,W. H., and Dineen, J. K. J. PP. 137-141. CSIRO Division of Animal Health, Melbourne.

LE-Jambre, L. F. (1981). Hybridizaion studies with Australian Haemonchus placie (place, 1893). Haemonchus contorus cayaguensis (Das and whitlock; 1960) and Haemonchus contorus (Rudolphi, 1803) from Louisiana. International Journal for Parasitology. 11: 323-330.

Le-Jambre, L. F. (1995). Relationship of blood loss to worm numbers, biomass and egg production in Haemonchus infected sheep. International Journal for Parasitology. 25: 269-273.

Lewis, W.H. and Elvin-Lewis, M.P.H. (1977). Medicinal botany Plants Affecting Mans Health. john Wiley and Sons, New York.

Lubega, G. W. and Prichard, R. K. (1991). Interaction of benzimidazole anthelmintics with Haemonchus contortus tubulin: binding affinity and anthelmintic efficacy. Exp. Parasitol. 73: 203-213.

Maciel S., Gimenez A. M., Gaona C., Wailer P. J. & Hansen J. W. (1996). The prevalence of anthelmintic resistance in nematode parasites of sheep in Southern Latin America: Paraguay.Veterinary Parasitology. 62: 207-212.

Maciel, M. V., MOrais, S. M., Bevilaqua,C. M. L., Camurca- Vasconcelo, A. L. F., Costa, C.T.C., Costro, C. M. S.(2006). Ovicidal and larvicidal of Melia azadarach extracts on Haemonchus contortus. Vet. Parasitol. 140: 98-104.

Magzoub, M., El Hassan, E. M. and Burger, H. J. (1990). Pasture infestation with trichostrongyles of camels in the Butana area of eastern Sudan. Tropical Animal health and Production. 22: 7-8.

Mahannop, P., Chaicumpa, W., Setasuban, P., Morakote, N., Tapchaisri, P., (1992). Immunodiagnosis of human trichinellosis using excretorysecretory (ES) antigen. Journal of Helminthology. 66: 297–304.

Malan, F. S. and Van Wyk, J. A. (1992). The packed cell volume and colour of conjunctivae as aids for monitoring Haemonchus contortus infestations in sheep. In: Proceedings of the South Africa Veterinary Association Biennial National Veterinary Congress (Eds: Grahamstown, South Africa, 7 - 10 September, p. 139.

Malczewski, A. (1970). Gastrointestinal helminths of ruminants in Polland: Pathogenesis and pathology of experimental Haemonchus contortus infection in lambs. Acta Parasitologica Polinica. 36: 399-415.

Manton, N. J. A.; Peacock, R.; Poynter, D.; Silverman, P. H. and Terry, R. J. (1962). The influence of age on naturally acquired resistance to Haemonchus contorus in lambs. Research in Veterinary Science. 3: 308-314.

Manueli P. R. (1996) Sustainable control of parasites in small ruminants--country paper: Fiji. In: Sustainable Parasite Control in Small Ruminants (Edited by LeJambre L. F. & Knox M. R.) pp. 92-97. ACIAR. Canberra.

Marco. J. A., Sanz-Cervera J.F. and Ocete. G. (1994). New germacranolides and eudesmanolides from North African Artemisia herba-alba. J. Nat. Prod., 57: 939-946.

Marie-Magdeleine, C., Hoste, H., Mahieu, M., Varo, H., Archimede. H . (2009). In vitro effects of Cucurbita moschata seed extracts on Haemonchus contortus. Veterinary Parasitology. 161: 99–105

Marie-Magdeleine, C., Mahieu, M. D'Alexis, S. Philibert, L. Archimede, H. (2010). In vitro effects of Tabernaemontana citrifolia extracts on Haemonchus contortus. Research in Veterinary Science in press.

Matika, O., Nyoni, S., Vanwy, K. B. J., Elasmus, G. J. and Baker, R. L. (2003). Resistance of Sabi and Dorper ewes to gastrointestinal nematode infections in an African semi-arid environment. Small ruminant Research. 47: 95-107.

Mccracken, R. O. and Sillwell, W. H. (1991). A possible biochemical mode of action for benzimidazole anthelmintics. Ivt. J. Parasitol. 21: 99-104.

McKerrow, J. H., Brindley, P., Brown, M., Gam, A. A., Staunton, C., Neva, F. A. (1990). Strongyloides stercoralis: identification of a protease that facilitates penetration of skin by the infective larvae. Experimental Parasitology. 70: 134–143.

Meaney. M. I., Fairweather, G. P. Brennan, A. B. Forbes. (2004). Transmission electron microscope study of the ultrastructural changes induced in the tegument and gut of Fasciola hepatica following in vivo drug treatment with clorsulon. Parasitol Res. 92: 232–241.

Meyer Jones, L.; Booth, M. H. and McDonald, L. E. (1977). Veterinary Pharmacology and Therapeutic. Fourth edition. The Iowa State University Press. USA oha 52, pp 1024-1032.

Michel, J. F. (1974). Arrested development of nematodes and some related phenomena. Advances in Parasitology. **14**: 355–397.

Michel, J. F. (1985). Strategies for the use of anthelmintics in livestock and their implications for the development of drug resistance. Parasitology. 90: 621-628.

Minja, M. M. J. (1989). Collection of Tanzanian medicinal plants for biological activity studies. In: Proceedings of the 7th Tanzania Veterinary Association Scientific Conference (Eds: Arusha, Tanzania, pp. 67-78.

Minja, M. M. (1994). Medicinal plants used in the promotion of animal health in Tanzania. Review of Science and Technology. 13: 905-925.

Mohamed, A. H, Eltahir, K. E, Ali, M. B, Galal, M, Ayed I. A, Hamid, O. A. (1999). Phytother Res.13:1.

Mohmoud A. Saleh, Mohamed H Belal; Gamal El Baroty.(2006). Fungicidal activity of Artemisia herba alba Asso (Asteraceae) Journal of Environmental Science and Health (B).41(3):237-244.

Monteiro, A. M., Wanyangu, S. W., Kariuki, D. P., Bain, R., Jackson, F. & &Mckellar, Q. A. (1998). Pharmaceutical quality of anthelmintics sold in Kenya. Veterinary Record. 142. 396–398.

Mottelib, A. A.; Haroun, E. M.; Magzoub, M. and El-Basheer, E. (1992). The effect of gastrointestinal parasites on blood picture in sheep and goats at Al-Gassim. Assiut Veterinary Journal. 55: 215-223.

Mugambi, J. M.; Bain, R. K.; Wanyangu, S. W.; Ihiga, M. A.; Duncan, J. L.; Murray, M. and Stear, M. J. (1997). Resistance of four sheep breeds to

natural and subsequent artificial Haemonchus contortus infection. Veterinary Parasitology. 69: 265-273.

Mugambi, J. M., Audho, J. O. and Baker, R. L. (2005). Evaluation of the phenotype performance of a Red Massai and Dorper clouded backcross resource population: natural pasture challenge with gastrointestinal nematode parasites. Small Ruminant Res. 56: 239-251.

Mulligan W.; Gordon H. McL., Stewart, D. F. and Wagland, B. M. (1961). The use of irradiated larvae as immunizing agents in Haemonchus contortus and Trichostrongylus colubriformis infections of sheep. Australian Journal of Agricultural Research. 12: 1175-l 187.

Murray, M. (1973). Local immunity and its role in vaccination. Veterinary Research. 10: 500-504.

Murray, M.; Robinson, P. B.; Grierson, C. and Crawford, R. A. (1979). Immunization against Nippostrongylns brasiliensis in the rat. A study on the use of antigen extracted from adult parasites and parameters which influence the level of protection. Acta Tropica. 36: 279-322.

Nadkarni, A.K., (1954). Indian Materia Medica,3rd Ed. Popular Prakashan, Bombay, India.

Nagvi, S. A. H., Vohora, S. B. and Khan, M. M.Y. (1991). Antibacteria, antifungal and anthelmintic studies of Artemisia scoparia Herbal Hungerica. 30: 54-60.

Nakhare, S. and Garag, S. C. (1991). Anthemintic activity of some essential oil of Artemisia paller wall. Ancient Sci. Life.10: 185-186.

Narayana, K., Setty, D. R. l., Roa, H. S., Kamalapur, P. N. (1976). A clinical and exeperimental study of Wopell in canine cestodiasis. Mysore Journal of Agricultural sciences. 10: 98-100.

Nari A., Salles J. Gil, A., Wailer, P. J. & Hansen J. W. (1996). The prevalence of anthelmintic resistance in nematode parasites of sheep in Southern Latin America: Uruguay. Veterinary Parasitology. 62: 213-222.

Neogi, N. C., Batiga, P. A. C. and Srivastava, P.K. (1964). Anthelmintic activity of some indigenous drugs. Indian J. Pharma. 26-37.

Newton, E. S. (1995). Progress on Vaccination Against Haemonchus contortus. International Journal for Parasitology. 25(11):1281-1289.

Nour, M. S., Magzoub, M. and Elnema, E. (2006). Efficacy of Randia nilotica methanol extract against schistosoma mansoni infection in mice. Sudan. Journal of Medical Science. 1 (2): 103-107.

Okan, D. E. and Enyerihi, U. K. (1977). Development and survival of Haemonchus contorus larvae on pasture in Ibadan. Tropical Animal Health and Production. 9: 7-10.

Okpekon, T.; Yolou, S.; Gleye, C.; Roblot, F.; Loiseau, P.; Bories, C.; Grellier, P.; Frappier, F.; Laurens, A. and Hocquemiller, R. (2004). Antiparasitic activities of medicinal plants used in Ivory Coast. Journal of Ethnopharmacology. 90: 91-97.

Oliver-Bever (1986).Medicinal plants in Tropical West Africa. Cambridge University Press, Cambridge. 375pp.

Oliveira, L. M.; Bevilaqua, C. M.; Costa, C.T.; Macedo, I. T.; Barros, R. S.; Rodrigues, A. C.; Camurça-Vasconcelos, A. L.; Morais, S. M.; Lima, Y. C.; Vieira, L. S. and Navarro, A. M. (2009). Anthelmintic activity of Cocos nucifera L. against sheep gastrointestinal nematodes. Vet Parasitol.159(1): 55 - 59.

Osman, O. A. (2001). Anticestodal activity of Sudanese medicinal plants against the poultry cestode. Rilettina tetragona. M.V.Sc. Thesis, University of Khartoum, Sudan.

Paolini, V., De La Farge, F., Prevot, F., Dorchies, P., Hoste, H., (2005). Effects of the repeated distribution of sainfoin hay on the resistance and the resilience of goats naturally infected with gastrointestinal nematodes. Veterinary Parasitology. 127: 277–283.

Paraud, C. and Chartier, C. (2003). Biological control of infective larvae of a gastro-intestinal nematode (Teladorsagia circumcincta) and a small lungworm (Muellerius capillaris) by Duddingtonia flagrans in goat feces. Parasitol. Res. 89: 102– 106.

Paraud, C.; Pors, I. and Chartier, C. (2004). Activity of Duddingtonia flagrans on Trichostrongylus colubriformis larvae in goat feces and interaction with a benzimidazole treatment. Small Ruminant Research. 55: 199 – 207.

Parker. R.J. and Palmer, B., (1991) Lack of anthelmintic effect of Calliandra calothyrsus in sheep . Australian veterinary journal. 68:309.

Peacock, R. and Poynter, D. (1980). Field experience with a bovine lungworm vaccine. In: Vaccines against Parasites (Edited bv Taylor A. E. R. & Muller R.), Symposia of the British Society for Parasitology, Vol.' 18: pp. 141-148. Blackwell Scientific Publications, Oxford.

Perry, B. D., Randolph, T. F., McDermott, J. J., Sones, K. R. & Thornton, P. K. (2002).Investing in animal health research to alleviate poverty. ILRI (International LivestockResearch Institute), Nairobi, Kenya, 148 pp. ISBN 9291461083.

Pessoa, L. M., Morais, S. M., Bevilaqua, C. M. L., Luciano, J. H. S. (2002). Anthelmintic activity of essential oil of Ocimum gratissimum Linn. and eugenol against Haemonchus contortus. Vet. Parasitol. 109: 59–63.

Piper, L. R. (1987). Genetic variation in resistance to internal parasites. Merino Improvement programs in Australia. Ed B. J. McGuirk. Melbourne, Australian Wool Corporation. PP 351-363.

Pradhan, S. I. and Johnstone, I. L. (1972). Heamonchus contortus heamotological changes in lambs during prolonged exposure to daily and weekly doses of infective larvae. Parasitology. 64: 153-160.

Preston, J. M. and Allonby, E. W. (1979). The influence of breed on the susceptibility of sheep of Haemonchus contortus infection in Kenya. Research in Veterinary Science. 26: 134- 139.

Prichard, R. K.; Hall, C. A.; Kelly, J. D.; Martin, I. C. A. and Donald, A. D. (1980). The problem of anthelmintic resistance in nematodes. Australian Veterinary Journal. 56: 239-250.

Raadsma, H. W.; Gray, G. D. and Woolaston, R. R. (1998). Breeding for disease resistance in Merino sheep in Australia. Review of Science and Technology. 17: 315-328.

Radhakrishnan, C.V. (1972). Host responses of worm-free Florida Native and Rambouillet lambs experimentally infected with Haemonchus contortus. American Journal of Veterinary Research. 33: 817-823.

Rahman, W. A. and Collins, G. H. (1990). Change in live weight gain, blood constituents and worm egg output in goats artificially infection with a sheep derived strain of Haemonchus controtus. British Veterinary Journal. 146 (6): 543-550.

Ransom, B. H. (1906). The life history of the twisted wire worm (Haemonchus contortus) of sheep, Cattle and other ruminants. Circular 93 U.S.D.A Bureau Animal Industry. PP. 1-7.

Rates, S. M. K. (2001). Plants as source of drugs. Toxicon. 39: 603–613.

Reid, J. F. S. and Armour, J. (1972). Seasonal fluctuations in inhibited development of gastrointestinal nematodes of sheep. Res. Vet. Sci. **13**: 225–229.

Richer, J. K., Sakanari, J. A., Frank, G. R., Grieve, R. B. (1992). Dirofilaria immitis: proteases produced by third- and fourth-stage larvae. Experimental Parasitology. 75: 213–222.

Riffkin, G. G. and Dobson, C. (1979). Predicting resistance of sheep to Haemonchus contortus infections. Veterinary Parasitology. 5 (4): 365-378.

Roberts, F. H. S. and O'Sullivan, P. J. (1950). Methods for egg counts and larval cultures for strongyles infecting the gastro-intestinal tract of cattle. Australian Journal of Agricultural Research. 27: 657-667.

Roberts, J. L. and Swan, R. A. (1982). Quantitative studies of ovine haemonchosis. The interpretation and diagnostic significance of the changes in serial egg counts of Haemonchus contortus in a sheep flock. Veterinary Parasitology. 9: 211-216.

Roger, S. W. P. and Sommerville, R. I. (1968). The infectious process and its relation to the development of early parasitic stages of nematodes. Advances in Parasitology. 6: 327-348.

Roger, S. W. P. and Sommerville, R. I. (1982). Enzymes in the exsheathing fluid by nematodes and their biological significances. International journal for parasitology. 12: 495-502.

Romjali, E.; Pandey, V. S.; Balubara, A.; Gatenby, R. M.; Verhulst, A. (1996). Comparison of resistance of four genotypes of rams to experimental infection with Haemonchus contotrus. Veterinary Parasitology. 65: 1-2, 127-137.

Roos, M. H. (1997). The role of drugs in the control of parasitic nematode infections: must we do without? Parasitology. 114: 137-144.

Ross, D. B. (1975). The effect of Fenbendazole on nematode parasite in experimental infected lambs. Veterinary Record. 96: 357-359.

Rothwell, J. T. and Sangster, N. C. (1996). The effects of closantel treatment on the ultrastructure of Haemonchus contortus. International Journal for Parasitology. 26:49-57.

Roy, B., Tandon, V. (1996). Effect of root tuber extract of Flemingia vestita, a leguminous plant, on Artyfechinostomum sufratyfex and Fasciolopsis buski: a scanning electron microscopic study. Parasitol. Res. 82: 248-252.

Said, M., (1969). Hamdard Pharmacopea of Eastern Medicine. Hamdard National Foundation, Karachi, Pakistan.

Salah. S. M. and Jaeger. A. K. (2005). Two flavonoides from Artemisia herba-alba Asso with in vitro GABAA-benzodiazepine receptor. J. Ethnopharmacol. 99: 145-146.

Salamn, S. K. and Duncan, J. L. (1984). The abomasal histology of worm-free sheep given primary and challenge infections of Haemonchus contortus. Veterinary Parasitology. 16: 43-54.

Saleh N. A. M., EI-Negoumy. S. I. and Abou-Zald M. M. (1987).Flavonoids of Artemisia judalca, A. monosperma and A. herba-alba. Phytochemistry. 26: 3059-3064.

Sam, M. S. and Katharina, A. J. (2005). Two Flavonoid from Artemisia herba alba Asso with in vitro GABA benzodiazepine receptor activity. Journal of Ethnoparmacology.99 (1):145-146.

Sangster, N. C., Davis, C. W. and Collins, G. H., (1991). Effects of cholinergic drugs on longitudinal contraction in levamisole-susceptible and -resistant Haemonchus contortus. Int. J. Parasitol. 21: 689-695.

SAS, (1998). SAS/STAT® user Guide version 6.12. SAS Inst. Inc., Cary, NC.

Satrija, F.; Nansen, P.; Bjorn, H.; Murtini, S. and He, S. (1994). Effect of papaya latex against Ascaris suum in naturally infected pigs. Journal of Helminthology. 68: 343-346.

Satrija, F., Retnani, E.B., Ridwan, Y. and Tiuria, R. (2001). Potential use of herbal anthelmintics as alternative antiparasitic drugs for small holder farms in developing countries. Livestock Community and Environment. Proceedings of the 10th Conference of the Association of Institutions for Tropical Veterinary Medicine, Copenhagen, Denmark.

Satyavati, G. V., M. K. Raina and M. Sharma. (1976). Medicinal Plants of India. Indian Council of Med. Res., New Delhi, India. Vol. I pp:201–06.

Schalling, H. D. F.; Horook, S. and Cornelissen, J. R. W. J. (1995). Comparison of tow enzyme immunoassay for the detection of Haemonchus contorus infection in sheep. Veterinary Parasitology. 57: 4, 329-338.

Schallig, H. D.; Van Leeuwen, M. A. and Cornelissen, A. W. (1997). Protective immunity induced by vaccination with two Haemonchus contortus excretory secretory proteins in sheep. Parasite Immunology. 19: 447-453.

Schmidt, E. E.; Suarez, V. H.; Busetti, M. R.; Babinec, F. J. (1994). Genetic variation of bovine resistance to gastrointestinal nematodes. Revista Argentina de Production Animal. 14: 1-2, 45-53.

Scrivner, L. H. (1964). Breed Resistance to Ostertagiasis in sheep. Journal of American Veterinary Medicine Association. 144: 883-887.

Shaik, S.A., Terrill, T.H., Miller, J.E., Kouakou, B., Kannan, G., Kaplan, R.M., Burke, J.M., Mosijidis, J.A., (2006). Sericea lespedeza hay as a natural deworming agent against gastrointestinal nematode infection in goats. Vet. Parasitol. 139: 150–157.

Sharma, L. D., H. S. Bahga and P. S. Srivastava. (1971). In vitro anthelmintic screening of indigenous medicinal plants against Haemonchus contortus (Rudolphi, 1803) of sheep and goats. Indian J. Anim. Res.5: 33–38.

Sharma, L. K., (1993). Anthelmintic efficacy of lantana capsules in crossbred cattle. Indian Veterinary Journal. 70: 459-460.

Shavulimo, R. S.; Rurangirwaf, F.; Ruvuma, F.; James, A. D.; Ellis, P. R.; McGuire, T. (1988). Genetic resistance to gastrointestinal nematodes, with special reference to Haemonchus contortus, in three breeds of goats in Kenya. Bulletin of Animal Health and Production in Africa. 36: 3, 233-241.

Shaw, D. J. and Dobson, A. P. (1995). Patterns of macroparasite abundance and aggregation in wildlife populations: a quantitative review. Parasitology. 111: 111-127.

Singh, Sheela, N.A. Ansari, M.C. Srivastava, M. K. Sharma and S. N. Singh. (1985). Anthelmintic activity of Vernonia anthelmintica. Indian Drugs. 22: 508–511.

Singh, R., Singhal, K.C. and khan, N.U. (1997). Antifilarial activity of Mallotus philibbensis lam of S. cervi (Nematoda: Filariodea) in vitro. Indian J. Physiol. Pharmacol. 41: 397-403.

Sivaraj S., Dorny P., Vercruysse J. & Pandey V. S. (1994). Multiple and multigeneric anthelmintic resistance on a sheep farm in Malaysia. Veterinary Parasitology. 55: 159- 165.

Smith, W. O. (1977). Anti-larval antibodies in the serum and abomasum mucus of sheep hyper-infected with Haemonchus contortus. Research In Veterinary Science. 22: 334-338.

Smith, W. D. and Christie, M. G. (1979). Haemonchus contortus some factors influencing the degree of resistance of sheep immunized with attenuated larvae. Journal of Comparative Pathology. 89:141-150.

Smith, W. D.; Newlands, G. F. J.; Smith, S. K.; Pettit, D. and Skuce, P. J. (2003). Metalloendopeptidases from the intestinal brush border of Haemonchus contortus as protective antigens for sheep. Parasite Immunology. 25: 313-323.

Soulsby, E. J. L. (1966). The mechanisms of immunity to gastrointestinal nematodes. In: soulsby, E. J. L. Biology of parasites, Emphasis on veterinary parasites. NewYork, London, Academic Press, PP. 255-276.

Soulsby, E. J. L (1982). Helminth, Arthopods and prtozoa of domesticated animals. 7th ed. Bailliere Tindall and cassell, London.

Soulsby, E. J. L. (1986). Helminth, Arthropods and protozoa of domesticated animals. 7th ed. Bailliere Tindall and Cassell, London.

Stewart, D. F. (1950). Studies on resistance of sheep to infestation with Haemonchus contortus and Trichostrongylus spp. and on immunological reaction of sheep exposed to infestation. II, the antibody response to infestation with Haemonchus contortus. Australian Journal of Agricultural Research. 1: 301-310.

Stewart, D. F. (1953). Studies on resistance of sheep to infestation with Haemmonchus contortus and Trichostrongylus spp., and on the immunological reactions of sheep exposed to infestation. The nature of the self-cure phenomenon. Australian Journal of Agricultural Research. 4: 100-117.

Stewart, D. F. (1955). Self-cure in nematode infestation of sheep. Nature. 167: 1273-1274.

Stoll, N. R. (1929). Studies of strongyloid nematode Haemonchus controus acquired resistance of hosts under natural reinfections out of doors. American Journal of Hygiene. 10: 284-418.

Subandriyo, Romjali, E.; Batubara, A.; Batubara, L. P.; Le-Jambre, L. F.; Know, M. R. (1996). Breeding for gastrointestinal nematode resistance of sheep in North Sumarta. Sustainable parasite control in small ruminants: an international workshop sponsored by ACIAR and held in Bogor, Indonesia, 22-25 April 1996. 134-140.

Sulieman, A. H. (1989). Proplem of sheep production in the Sudan. The international Symposium on the development of animal resourcesin the Sudan. pp383-387.

Suliman, A. M. and Jackson, J. K. (1959). Sudan Silva: 9 pp. 1-4.

Tadesse, D.; Eguale, T.; Giday, M. and Mussa, A. (2009). Ovicidal and larvicidal activity of crude extracts of Maesa lanceolata and Plectranthus punctatus against Haemonchus contortus. J. Ethnopharmacol. 122(2): 240 - 244.

Takhtajan, A. (1969). Flowering plants: origin and dispersal, Oliver and Boyd, Edinburgh, 310 pp.

Tandon, V., Pal, P., Roy, B., Rao, H. S. P., Reddy, K. S., (1997). In vitro anthelmintic activity of root tuber extract of Flemingia vestita, an indigenous plant in Shillong, India. Parasitol. Res. 83: 492-498.

Tangpu, V. and Yadav, A.K. (2004). Anticestodal activity of Trifolium repens extract. Pharmaceutical Biology. 42: 1-3.

Tariq. K. A., Chishti M. Z., Ahmada F., Shawl A. S. (2009) Anthelmintic activity of extracts of Artemisia absinthium against ovine nematodes. Veterinary Parasitology. 160: 83–88.

Taylor, M. A., Coop, R. L., Wall. R. L. (2007). Veterinary Parasitology, third edition – Blachwell bublishing Ltd.

Terrill, T. H.; Mosjidis, D.A.; Shaik, S. A.; Miller, J. E.; Burke, J. M.; Muir, J. P.; Wolfe, R. (2007). Effect of pelleting on efficacy of Sericea lespedeza hay as a natural dewormer in goats. Veterinary Parasitology. 146: 117-122.

Thamsborg, S. M., Roepstorff, A.and Larsen, M. (1999). Integrated and biological control of parasites in organic and conventional production systems. Veterinary Parasitology. 84: 169-186.

Thamsborg, S.M., Mejer, H., Bandier, M., Larsen, M. (2003). Influence of different forages on gastrointestinal namatode infections in grazing lambs. The 19th International Conference for the Advancement of Veterinary Parasitology, New Orleans, Louisiana, USA, 10.-14.

Theimpont, D.; Vanparids, O. F .J.; Raeymackers, A. H. N.; Vandenbwrk, J.; Demon, P. J. A.; Allewjn, F. T. N.; Masshoon, R. P. R. Nieregeers, G.

T. C.; Schellekens, K. H. L. and Janssen, F. A. T. (1966). Tetramisole (R8299) a new potent broad spectrum anthelmintic. Nature. 209: 1084.
Theimpont, D.; Rochett, F. and Vanparids, O. F. J. (1986). Diagnosis Helminthiasis by Coprological Examination. 2nd Edition, Janssen Foundation, Belgium. PP 47-63.
Tietz, N.W. (1982). Fundamentals of clinical chemistry. Saunders, W.B. Press, 2nd edition, pp. 1194.
Trease, G.E. and Evans, W.C. (1978). Pharmacognosy text book 11th edition, p. 107. Bailliere Tindall Publishers.
Uphoff, J. C. J. (1968). Dictionary of economic plant,2nd edition, p. 63, Verleg Ven j. Cramer Publishers, W. Germany.
Urquhart, G. M.; Jarrett, W. F.; Hjennings, F.W.; Mcintyre, W. I .M.; Mulligan, W. and Sharp N. C. C. (1966). Immunity to Haemonchus contortus infection, relationship between age and successful vaccination with irradiated larvae. The American Journal of Veterinary Research. 27, 1642-1648.
Urquhart, G. M.; Armour, J.; Duncen, J. L.; Dunn, A. M. and Jennigs, F. W. (1987). Veterinary Parasitology. Harlow, U.K.; Longman Group 286pp.
Urquhart, G. M., Armour, J., Duncan, J. L., Dunn, A. M., Jennings, F. W. (1996). Veterinary Parasitology, second ed. Blackwell Science, London.
Van Wyk, J. A. and Malan, F. S. (1988). Resistance of field strains of Haemonchus contorus to ivermectin, closantel, rafoxanide and benziidazole in South Africa. Veterinary Record. 123: 226-228.
Van Wyk, J. A. and Bath, G. F. (2002). The FAMACHA© system for managing haemonchosis in sheep and goats by clinically identifying individual animals for treatment. Veterinary Research. 33: 509 - 529.
Van Wyk, J. A.; Bath, G. F. and Malan, F. S. (1998). The need for alternative methods to control nematode parasites of ruminant livestock in South Africa. World Animal Review.91.
Varshney, I.P. and Jain, V. (1979). Indian Journal of Pharmaceutical Sciences, pp. 122-123.
Varshney, J.P., Pramaod, V., Svivistava, H.C. and Singh, P.P. (1977). The Indian Journal of Pharmacy, p. 125-128.
Vatta, A. F., Krecek, R. C., Letty, B. A., van der Linde, M. J., Motswatswe, P. W. & Hansen, J. W. (2002). Effect of nematode burden as assessed by means of faecal egg counts on body condition in goats farmed under resource-poor conditions in South Africa. Veterinary Parasitology. 108: 247-254.

Veglia, F. (1915). The anatomy and life history of the Haemonchus contorus. Report of Veterinary Research South Africa. 3/4: 347-500.

Vercruysse, J. (1985). The seasonal prevalence of inhibited development of Haemonchus contortus in sheep in Senegal. Vet. Parasitol. **17**: 159–163.

Voller, A. and Desavigny, D. (1981). Diagnostic serology of tropic parasitic diseases. Journal of Immunological Methods. 46: 1-29.

Waller, P. J. (1986). Anthelmintic resistance in sheep nematodes. Agricultural Zoological Reviews. 1: 333-373.

Waller. P. J. (1993). Towards sustainable nematode parasite control of livestock. Veterinary Parasitolog. 48: 295-309.

Waller, P. J. (1997). Nematode parasite control of livestock in the tropics/subtropics: the need for novel approaches. International Journal for Parasitology. 27: 1193-1201.

Waller, P. J. and Thomas, R. J. (1975). Field studies on inhibition of Haemonchus contorus in sheep. Parasitology. 71: 285-291.

Waller P. J. and Faedo M. (1996). The prospects for biological control of the free-living stages of nematode parasites of livestock. International Journal for Parasitology. 26: 915- 925.

Waller P. J. Echevarria F.. Eddi C., Maciel S., Nari A. & Hansen J. W. (1996). The prevalence of anthelmintic resistance in nematode parasites of sheep J.in Southern Latin America: general overview. Veterinary Parasitolog. 62: 181-187.

Walls, K. W. and Schantz, P. M. (1986). Helminth Diseases. In: Immunodignosis of parasitic Diseases, vol.1. Harcourt Brace Jovanoich Academic press: London and Newyork.

Wanyangu S. W.; Bain R. K., Rugutt M. K., Nginyi J. M. & Mugambi J. M. (1996). Anthelmintic resistance amongst sheep and goats in Kenya. Preventive Veterinary Medicine. 25: 285-290.

Waruiru, R. M.; Ngotho, J. W. and Mukiri, J. G. (1998). Multiple and multigeneric anthelmintic resistance on a sheep farm in Kenya. Tropical Animal Health and Production. 30: 159-166.

Watt, J. M. and Breyer-Brandwijk, M.G., (1962). The Medicinal and Poisonous Plants of Southern and Eastern Africa, 2nd edition. Livingstone Ltd., Edinburgh, London, pp. 1457.

Weiss, N.; Hussain, R. and Ottesen, E. A. (1982). 1gE antibodies are more specific than 1gG antibodies in human onchocerciasis lymphatic filariasis. Immunology. 45: 129-137.

Williamson, J. F. (1995). Effect of albendazole controlled release capsules on immunity to internal parasites in sheep. Newzealand Veterinary Journal. 43: 3-127.

Wilson, K. and Grenfell, B. T. (1997). Generalized linear modelling for parasitologists. Parasitology Today. 13: 33-38.

Windon, R. G.; Dineen, J. K. and Kelly, J. D. (1979). The segregation of lambs into "responders" and "non-responders": response to vaccination with irradiated Trichostrongylus colubriformis larvae before weaning. International journal of Parasitology. 10: 65 – 73.

Wood, I. B.; Amaral, N. K.; Bairden, K.; Duncan, J. L.; Kassai, T., Malone, J. B.; Jr Pankavich, J. A.; Reinecke, R. K., Slocombe, O. and Taylor, S. M. (1995). World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). Veterinary Parasitology. 58: 181-213.

Woolaston, R. R. and Baker, R. L. (1996). Prospects of breeding small ruminants for resistance to internal parasites. International Journal for Parasitology. 26: 845-855.

Xiaojun, L.i.; Aifang, D.u.; Weiming, Cai; Yuhui, Hou; Linhai, Pang. and Xiang, Gao . (2007). Evaluation of a recombinant excretory secretory Haemonchus contortus protein for use in a diagnostic enzyme-linked immunosorbent assay. Experimental Parasitology. 115: 242–246.

Yates, D. M., Portillo, V. & Wolstenholme, A. J. 2003. The avermectin receptors of Haemonchus contortus and Caenorhabditis elegans. International Journal for Parasitology. 33: 1183-1193.

Zajac, A. M.; Krakoka, S.; Herd, R. P. and McClure, K. E. (1990). Experimental Haemonchus contortus infection in three breeds of sheep. Veterinary Parasitology. 36: 3/4, 221-235.

Zaroug, I. M., Nugul, A. D., Bashir, A. K. and Mageed, A. A. (1988). Evaluation of Sudanese plant extracts as mesquites larvicides. Int. J. Crud Drug Res. 26: 71.

ملخـص الأطـروحـــــة

أجريت هذه الدراسة لتقييم الأثر المضاد للديدان داخل الحيوان وفي المعمل لنباتي اللالوب والشـيح ضـد دودة الهيمونكس في الضان.

تم استخدام 28 حمل تتراوح أعمارها بين 4-5 شهور من نوع الحمري للدراسة داخل الحيوان. تم تغذيتها بالذرة وقشـر الفول السوداني. أعطيت جرعتين من أي من مضادات الديدان والمضادات الحيوية ومضادات الكوكسيديا لمعالجتها من أي عدوي محتملة. تم تقسيم هذه الحيوانات عشوائياً إلي 7 مجموعات تحتوي كل مجموعة علي 4 حملان. ست مجموعـات تمت تعديتها فمياً بجرعة واحدة تحتوي على 3000 طور يرقي ثالث من دودة الهيمونكس. مجموعتان أعطيت كل منهمـا فمياً 3جم/كجم من وزن الجسم من المستخلص المائي لنبات الشيح (مجموعة أعطيت قبل يوم من العدوى والثانية بعد 25 يوم من العدوى). بالمثل أعطيت كل من المجموعتين الثالثة والرابعة 9 جم/كجم من مسـتخلص لـب ثمـرة اللالـوب (مجموعة أعطيت قبل يوم من العدوى والثانية بعد 25 يوم من العدوى). المجموعة الخامسة عولجت بالالبندازول 25 يوم بعد العدوى. المجموعة السادسة أعديت وتركت من غير علاج للمقارنة. أما المجموعة السابعة فقد تركت من غير عدوي ومن عير علاج للمقارنة. تم أسبوعيا جمع عينات البراز والدم لمعرفة عدد البيوض وتقييم مكونات الدم. الحيوانات المعدية والمعالجة تم ذبحها بعد 6 أسابيع من العدوى لمعرفة عدد الديدان البالغة في المنفحة.

أوضحت النتائج أن الحيوانات المعدية أظهرت انخفاضاً في تركيز الهيموغلوبين, مكداس الدم, عدد كريات الـدم الحمراء والأوزان الحية المكتسبة مقارنة بالحيوانات الغير معدية. لم تظهر أي أعراض سمية لنباتي الشـيح واللالـوب. الحيوانات التي عولجت بالشيح واللالوب قبل العدوى أظهرت انخفاضاً طفيفاً في عدد البيض بالجرام والعدد الكلي للديدان مقارنة بالحيوانات المعدية الغير معالجة. الحيوانات التي عولجت بعد العدوى أظهر اللالوب فيها فعالية 69.9% وأعلـي نسبة إنخفاض في البيض في اليوم ال14 بعد العلاج 69.6% مقارنة ب62.5% و61% علي التـوالي للشـيح و97.4% و98.5% علي التوالي للالبندازول (5مج/كجم).

الحيوانات التي تمت معالجتها بالشيح واللالوب قبل العدوى أظهرت زيادة غير معنوية في تركيز الهيموغلوبين, مكداس الدم وعدد كريات الدم الحمراء. أما التي عولجت بعد العدوى قد أظهرت زيادة معنوية فـي هـذه المعـايير بعـد أسبوعين من العلاج. لم تظهر أي اختلافات معنوية في عدد كريات الدم البيضاء, الحمضية, اللمفاوية, المتعادلة والأوزان الحية المكتسبة في الحيوانات التي عولجت قبل أو بعد العدوى مقارنة بالتي اعديت ولم تعالج.

للدراسة في المعمل تم اختبار المستخلصين المائي والكحولي للشيح واللالوب بتركيز 10,5, 25 و50 ملج/مـل علي الديدان البالغة والطور اليرقي الثالث لدودة الهيمونكس. مستخلصا النباتين اظهرا تأثيراً مضاداً علي الديدان البالغـة. أوضح الشيح بتركيز 50ملج/مل تأثيراً معنوياً مضاداً اكبر للديدان في حالة المستخلص الكحولي (100% نسبة موت بعد2 ساعات) مقارنة بالمستخلص المائي (90% نسبة موت بعد6 ساعات). أما المستخلص المائي للالوب فقد اظهر فعاليـة اكبر (100% نسبة موت بعد6 ساعات) مقارنة بالمستخلص الكحولي (70% نسبة موت بعد6 سـاعات). المستخلصـان المائي والكحولي للشيح واللالوب إضافة إلي الالبندازول ليس لديهم أي تأثير على الأطوار اليرقية.

اختبرت التركيبة العالية الدقة لبعض الديدان الغير متحركة بعد معالجتها بهذه النباتات تحت المجهر الالكتروني. لوحظت التغيرات في عضلات الجدار الخارجي والقناة الهضمية والجهاز التناسلي. هنالك تضخم في الألیـاف العضـلية والخمايل الدقيقة للأمعاء أصبحت متفرقة بعضها فقد والآخر غير منتظم ومتقلص في الحجم. الغدد المحية بالجهاز التناسلي أظهرت وجود عدد من الفراغات. المايتوكوندريا بها استطالة في الشكل بعضها صغير في الحجم والبعض الآخر متورم.

أوضحت النتائج أن لنباتي اللالوب والشيح فعالية ضد دودة الهيمونكس داخل الحيوان وفي المعمل ولكن لـيس لديهما فعالية عند إعطائهما قبل العدوى.