# *Effect* of *Piper abyssinica and Jatropha curcas* Against Experimental *Haemonchus contortus* Infection in Desert Goats

By

Nawal Abd El Raheem Mohamedo Adam

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Supervisor

Dr. Samia M.A. El Badwi,

Department of Medicine, Pharmacology and

Toxicology, Faculty of Veterinary Medicine,

University of Khartoum.

Co-Supervisor

Dr. Adam Dawoud Abbaker,

Academic Affairs,

Nyala University.

Faculty of Veterinary Medicine,

University of Khartoum,

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# DEDICATION

*To the soul of my lovely sister Ibtihal*

*who dedicated her life to me,*

*To my parents to whom*

*I am indebted - God blessed them,*

*To my husband*

*who encouraged and supported me in this work.*

<div align="right">

*Nawal*

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# *ACKNOWLEDGMENTS*

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# ABSTRACT

The present study was run to evaluate the anthlmintic activity of *Piper abyssinica and Jatropha curcas* against experimental  *H. contortus* infection in desert goats. All the groups (A,B,C and D) were orally infected with a single dose of 125 third stage larvae of *H. contortus.* The powdered seed of *Piper abyssinica* (dosed at 500 and 1000mg/kg b.wt. for two consecutive days) produced 18.6 and 57.4% efficacy rates respect ively.

The powdered seed of *Jatropha curcas* (dosed 250 and 500mg/kg bwt. for two consecutive days) produced 8.1 and 43.3% efficacy rates respectively. The powdered seed of *Piper abyssinica* dosages showed a significant reduction in worm numbers compared to the control group. The powdered seed of *Jatropha curcas*  dosages also showed a significant reduction in worm number when compared to the infected control.

The effectiveness of powdered seeds both of *Jatropha curcas*  and *Piper abyssinica* was weaker than that produced by Ivermectin at a dose of 200 μg/kg. b.wt, comparatively the effectiveness of powdered seed of *Piper abyssinica* was better than that of  *Jatropha curcas.*

The clinical signs in all infected groups  were manifested by dullness, weakness, emaciation, diarrhoea and  pale visible mucous membranes. After treatment, there were slight histopathological changes in *Piper abyssinica* groups, slight congestion in liver, small intestine, kidney, severe congestion and haemorrhage in abomasums and haemosidrosis in the spleen.

In groups that were treated with *Jatropha curcas* there was slight congestion in kidney and abomusum  and the spleen showed haemosiderin deposit.

*H. contortus* eggs were detected in faces at days 17 and 18 post infection with maximum shedding at days 19 and 25. There were decreases in Hb, PCV and RBCs in the infected control while the values of these haematological parameters are significantly increased in the treated groups of the two plants. The biochemical alterations of infected control were characterized by reduction in total protein, albumin and iron concentrations  whereas these parameters were significantly increased in the treated groups of the two plants including the group that received Ivermectin.

# خلاصة

أجريت هذه الدراسة لتقيّيم تأثير نباتين من النباتات الطبية هما نبات الشاومكادة وحبة الملوك ضد دودة الهمونكس في الماعز الصحراوي، حيث تمت الاصابة تجريبياً بكل مجموعات الماعز فموياً بجرعة واحدة باليرقات الثوالث لدودة الهمونكس بمعدل 125 يرقة للكيلو جرام الحي.

اجرعت هذه المجموعة بدرة بذور نبات الشاومكادة بالجرعات 500 ملجم، و1000 ملج لكل كجم من وزن الحيوان لمدة يومين متتاليين، هذه الجرعات اظهرت فعالية بلغت 18.6 و 57.4% ، ولم تظهر أي آثار سامة على الماعز. استعمل نبات حبة الملوك بالجرعتين 250 ملجم، و500 ملي جرام لكل كيلو جرام من وزن الحيوان على التوالي لمدة يومين متتاليين، وكانت الفعالية 8.1 و 43.3% على التوالي.

أظهرت حبة الملوك فعالية بلغت 8.1 و 43.3% مقارنة بعقار الافيرمكتين والذي اظهر فعالية عالية بلغت 99.5% بالجرعة 200 جرام كيلو من وزن الحيوان. اثبتت الدراسة الحالية أن نبات الشاومكادة ذو تأثير علاجي على دودة الهمونكس افضل من نبات حبة الملوك. الاعراض انحصرت في الهزال والضعف العام مع شحوب في الاغشية المخاطية.

التغيرات النسيجية في الكبد والكلي نلاحظ بهما نزيف حبري مع وجود احتقان بالاضافة للتغيير الدهني في الكبد، وتخضب الطحال بالهيموسدرين. التغييرات الكيميائية تمثلت في انخفاض في تركيز البروتين الكلي في بداية العدوى مع زيادة طفيفة بعد المعالجة. زيادة في تركيز الالبيومين وانخفاض في تركيز اليوريا في مصل الحيوانات. ارتفاع في تركيز الكرياتينين، وارتفاع في تركيز البليروبين، وارتفاع الحديد في مصل الحيوانات المعالجة.

التغيرات النسيجية تنحصر في الكبد. نلاحظ وجود الاحتقان والنزف وتغييرات دهنية على الكبد، في المنفحة احتقان ونزف، وجود الديدان البالغة بداخل المنفحة أو ملتصقة على الغشاء المخاطي، الامعاء الدقيقة والكلى بها احتقان مع وجود نزيف حبري في الكلى، تخضب الطحال بالهيموسدرين. تم رصد بيوض الديدان في براز الحيوانات المريضة في اليوم السابع عشر والثامن عشر من العدوى مع حدوث الحد الادنى للاخراج في اليوم التاسع عشر من العدوى

التغييرات الكيميائية بعد المعالجة تشمل ازدياد في تركيز البروتين الكلي ارتفاع طفيف في تركيز الالبيومين، انخفاض في تركيز اليوريا، ارتفاع في الكرياتينين، انخفاض في تركيز البلوربين وارتفاع الحديد في مصل الحيوانات المعالجة.

# INTORDUCTION

Plants has been considered as an efficient source of necessary drugs for many years and their active contents such as alkaloids, tannins, glycosides, saponins and flovonoids has been extracted and used in medication of various diseases.

Currently medicine industry became more developed and the progressing in plant chemistry and pharmacognosy has result in an obvious improvement in herbal medicine. The modern medicine became costly for a large proportion of the Sudan inhabitants, to set thus, efforts must be exerted to make Sudan self sufficient in this industry and this will be achieved by encouraging the production and manufacture of local medical by maintaining pharmaceutical preparation in a competent manner.

*Haemonchus contortus* was found to have a serious impact in the production of sheep and goats in the tropics **(Allonby, 1975; Schillhorn, 1978).** It caused an annual loss of more than 26 million dollars in sheep and goat production in Kenya as an example **(Allonby, and Urquhart 1976).** The loss estimation of sheep and goat production was about 11.3% in Kenya **(Graber, 1965)** 11% in Nigeria **(Schillhorn, 1973)** and over 40 million dollars in South Nigeria **(Akorejola *et al.* 1979).**

The loss in production caused by *H. contortus* and all the gastrointestinal parasites is due to high mortality among infected animals specially lambs and for example**, Eysker and Ogunsusi (1980)** reported over 30% of ewes died in extremis due to Gastrointestinaltract parasite mainly *H. contortus* and the cost of the anthlmentic drugs and *H. contortus* affects the general body

condition and reduced production of infected animal **( Eysker and Ogunsusi, 1980 and Fabiyi, 1987).**

Many antinematodal drugs such as Albendazole, Levamisole, Haloxon, Ivermectin and the modern medicines are very expensive.

**The objective of this study:**

1. To examine the activity of the two plants *Piper abyssinica*, *Jatropha curcas*, against experimental *H. Contortus* infection in goats

2. To determine the efficacy of the two plants compared with Ivermectin as reference anthelmintic drug.

3  To compare the  result obtained by the doses used of two plants as

anthelmintics.

CHAPTER ONE

LITERATURE  REVIEW

## 1.1. Parasitological review

*1.1.1  Haemonchus contortus*

*Haemochus contortus* has long been reported as a common parasite, It is found in the abomasum of sheep and goats

**1.2 Classification**:

Classified by **Soulsby, 1982;** and **Urquhart,** *et al***, 1996**  as follows

Phylum: Nematohelmenthis

Class: Nematoda

Sub Class: Secerrentea

Order: Strongylida

Supper Familly: Trichstrongyloidea

Family: Trychstronglidae

Genus: Haemonchus

Species: contortus

The host parasite  relationship appears to be more or less adopted between species of the genus Haemonchus and their ruminent hosts goats, sheep and some wild ruminent are infected with *H. contortus* while cattle are usually infected with *H. placei* and camels are infected with *H. Longistipes*. In spite of *H. contortus* and *H. placei*  infect hoterologous hosts, the establishment of these parasites on the other hosts is variable. However, infection of goats with *Haemonchus longistipes* showed pathological finding similar to that of *H. contortus* (Arzoun *et al.* 1983).

**1.1.2 Life cycle of *Haemonchus controtus*:**

The life cycle of *H. contortus* is typical to its super family Trichostrongylidae which have a direct life cycle **(Soulsby, 1982).** The development alternates without any intermediate host. The life cycle of this parasite is divided into two phases. The first one includes the extra host stages, the free living stages such as eggs and first, second and third larval stages (periparasitic or non parasitic phase). The second one is the parasitic phase which includes fourth and fifth larval stages and mature worms. The life cycle starts when mature female worms lay fertile eggs in the abomasal lumen. The eggs pass through alimentary tract and appear in faeces. The female of *Haemanchus contortus* lays about 5000-15000 eggs per day **(Hansen and Perry, 1994).** At the beginning of egg production the female lays a small number of eggs per day and then increase rapidly to reach a peak 25-30 day post infection. The egg of *Haemonchus contortus* is a strongyle shaped egg containing about 16-32 divided cells. It embryonates and hatches to the first larvae in favorable environmental condition specially moisture and temperature **(Hansen and Perry, 1994).** The first larvae moult and exchange their cuticle sheath to the second larvae. The first and second larvae had arbaditoform osoephagus and live free.

The second larvae moult to the third ones, the infective stage. It is non feeding, motile, has survival ability in different condition because of the cuticle sheeth the larvae migrate horizontally and vertically according to changes in temperature, moisture and humidity during the day, They migrate up and down plates of grass according to the amount of moisture. For example when the dew is on the grass or there is a rain fall, the larvae migrate to the top of the herbage, but following evaporation larvae will

migrete down the herbage and even into the soil **(Hansen and Perry, 1994)** This behaviour is a survival mechanism of the parasite before finding a suitable host.

The larvae invade the host orally through contaminated food and water. After ingestion the infective larvae reach the rumen where they exsheath and move to the abomasums and invade the mucosa. The fourth larval stage appears three to four days post ingestion. On special occasions, the development of the fourth larvae is inhibited for a period of time reaching three to four months before they resume their development later. The fourth larvae stages moult to fifth larval stage which develops to adult worm **(Soulsby, 1982).**

The prepatent period of *H. contortus* in goats may reach 18 days, usually it takes different value acorroding to the environmental condition, host immunity and age**. (Rahaman and Collins 1990)** studied the establishment of the parasite in goats. They found that all worms completed the third moult to the fourth by the fourth day post infection, the fifth larval stage and the adult worm started to migrate from the  abomasal mucosa to the leumen at day seventh to day eleventh post infection. The worms became adults at day eleventh post infection. Furthermore, 13.2% of female worms had eggs in their uteri by eighteenth day and more than half of the females had eggs in their uteri after the third week post infection.

### 1.1.3  Clinical signs

The clinical signs of Haemonchosis have been intensively described in sheep, cattle and goats **(Fabiyi *et al*., 1979; Eysker and Ogunsusi, 1980; Jubb  et al., 1985; Al-quaisy et al., 1987; Blood and Radostitis, 1989; Taylor *et al*., 1990; Abakar, 1996; Omar, 1999); Rahman and Collins, 1990),** infected animal showed anemia, hypoproteianemia, sub mandbular

odema is a common feature. Constipations or diarrhoea may develop when the disease is complicated by other gastrointestinal parasite in severe cases death may occur without clinical signs.

### 1.1.4  Drugs used against *H. contortus*

Many anathematic drugs have bean used for many years and their affections is initially related to their therapeutic index, spectrum of activity and their efficiency against both mature and immature worms **(Baker and Walters, 1971)**. The most important are the nematocides  group such as Thiabendazole,  Penzimidazole, Levamisole, Morantel and Naphthalphos. The trematodocides like closantel, clioxanide, rafoxanide and nitroxynil had high efficiency against *H. contortus*. The oral administration of albendazole at dose rate of 7.5 mg/kg and closantel at a dose rate of 10 mg/kg reduced feacal egg count by 79-93% and 99.52% **(Yadav   *et al*., 1993).** The authors stated that both fenbendazole and thiophanate reduced faecal egg count and total adult worm burden. Phenothiazine has been also used for Haemonchosis treatment. Ivermactin,  moxidectin, cyclectin and duramectin effective against haemonchosis. **Hansen and Perry, (1994)** summarized the common drugs that treat *H. contortus* in animals (Table 1)

# Table (1)  The common drugs used against Haemonchosis

| Generic name | Route of administration | Dose rate (mg/kg) |
|---|---|---|
| Albendazole | Oral | 5-7.5 |
| Cambendazole | Oral | 20-25 |
| Febantel | Oral | 5-10 |
| Fenbendazole | Oral | 5-7.5 |
| Mebendazole | Oral | 12-5 |
| Oxfendazole | Oral/intraruminal | 4.5-5 |
| Oxibendazole | Oral | 10-15 |
| Parbendazole | Oral | 20-30 |
| Thiabebndazole | Oral | 44-110 |
| Thiophanate | Oral | 50-80 |
| Tetramisole | Oral | 15 |
| Levamisole | Oral/spot on/subcutaneaus | 7.5-9 |
| Coumaphos | Oral/Feed | 8-15 |
| Haloxon | Oral | 40-50 |
| Naphtalophos | Oral | 30 |
| Trichlorfon | Intramuscular/subcutaneous | 10-15 |
| Morantel | Oral | 10 |
| Pyrantel | Oral | 25 |
| Ivermectin | Oral/subcutaneous/spot on | 200-500 mcg/kg |

## 1.2  Plants review

### 1.2.1  Plants with general anthelmintic effects

**Oliver – Bever (1986)** has briefly gone on account of West African medicinal plants that can eradicate helminthic parasites through lysis.

Some kind of plants contain protuelytic enzymes like bromelain from *Ananas comosus*, Calotropain from *Calotropis procera*, papain from *Carica papaya* all these enzymes digest worms. The same author reported that the wax – like substances from the seed of Caat of *Bixa orellane* paralyze intestinal parasites.

> There is plants contain glycosides with resins in a form of genin. The mode of action of these plants due to the genin, some plants have anthelmintic activity like *Cucurbita maxima* and *C.pepo* **(Watt and Breyer – Brand Wijk, 1962).**

The leave juice of the plant *Melia azedrach* (Meliaceae) is used as anthelmintic, diuretic and the seed is used for rheumatism **(Chopra, *et al*., 1956).**

*Withenia somnifera* (Solanaceae) the local name is Sim El Far or Sim El Firakh. The medical use of this plant as a phrodisiac, tonic, anthelmintic and narcotic by traditional medicine practitioners. It described also as an adaptogen, which enhances survival during stress **(Singh *et al*., 1982).** Withaferin, whithnolides, steriodal lactones withasomine, pyrazole al-kaloids, visamine tropine, volatile oil were investigated by some authors **(Covello and Ciampa, 1960**; **Khanna    *et al.,* 1961; Abraham    *et al.,* 1975).** The dried seeds of *Azidirachta indica*, the local name is Neem tree (Meliaceae) are used in India and Serilanka as insectcide to control pests in houses. The oil extract from the seed of *A. indica* is used as anthelmintic agent in man and animals, as aparasiticde for ringworm scabies and other skin conditions **(Fernando, 1982).** The major active ingredients in Neem

seed are azadirachtin, vepaol, lsovepaol, nimbidin and gedunin **(Zanno  *et al*.; 1975)*, *Sankaram *et al,* 1986; Khalid *et al*., 1989).**

The latex of *ficus carica* (Moraceae) is used as an anthelmintic and for coagulation of milk **(Wasim and Abdul – Malik, 1994).**

<center>1.2.2 Plants with antinematodal effect</center>

There is many plants that contain alkaloids the active ingredients against nematode, such as Echitamine from *Alstonia boonei,* which acts against *loaloa filariasis*. Flavonoids found in *Citrus acida* and *Albizia lebbeck* also have antinematodal properties **(Oliver – Bever, 1986).**

*Artemisia herba alba*, *A. cina* and *A. afara* (Asteraceae) used in subtropical countries as anthelmintics particulary for hook worm. They are used also as carminative, and antispasmodics **(Watt and Breyer Brand – wijk, 1962; Idris, *et al*., 1982).** The aerial parts of the former species contain sartonin, stigmstrol, B-sitosterol, flavonoids, sesquiterrpene and lactones **(Khafagy, *et al.,* 1971; Segal *et al.,* 1977).**

*Horrisonia abyssinica* (Simaroubacoae) was used as anthelmintic against oxyuris and ascarids (Watt and Breyer – Brand Wijk 1962), some African tribes inhale at the smoke from the burning root bark for ancylostomyasis. The volatile oil obtained from *Hybissious officinalis*  (Labiateae) L.,. growing in Egypt is effective against *Ascaridia galli* in chicks **(Sayed *et al*., 1978).**

Alcoholic extracts of the rhizomes of *Alpinia galanga* (Scitaminaceae *Tehprosia pumpuria* (Leguminosae), and *Zingiber zerumbeth* (Zingiberaceae) and tender leaves of *Morinda citrifolia* (Rubiaceae) and *Andrographis spaniculate* (Acanthaceae), the bark of *Cinnamonum zeybanicum* (lauraceae), *Citrus decumona* (Cucurbitaceae) and seeds of *Hydnocarpus withtiana* (Flacourtiaceae) showed a high in vitro anthelmintic

activity against human *Ascaris lumbricoides* **(Raj, 1975).** The same author demonstrated a high in vitro anthemantic activity against *A. lumbricoides* with alcoholic extract from the bark of *Albizia lebbeck* (Mimosaceae), bulb of *Allium sativum* (Umbelliferae), rhizomes of *Zingiber zerumbeth* (Zingiberaceae), *Punica granatun* (Punicaceae) and leaves of *Morinda citrifolia* (Rubiaceae). **MuKherjee and Suku, (1978),** found that the water extract of *Andrographis spaniculata* (Acanthaceae) was tested both in vitro and in vivo against filarial worms in dogs and the results were encouraging.

The leaves of *Ritchiea capparoides* are used in Senegal as Anti venomous and anti – filarial, and the roots plus leaves are used externally in the treatment of snake bites, beside these effects the roots and twigs are used as plaster on the enlarged cervical lymph nodes in patients suffering from Gambiense trypanosomiasis **(Kerharo and Adam, 1974).**

The oil of *Chenopodiutn ambrosioide* (Chenopodiaceae) was found to be effective against adults strongyles in the horse and ascarids in swine, dogs and cats but it is toxic to those species of animals **(Hall and Foster, 1918).**

The alcoholic extract of the aerial parts of *Ethulia conyzoides* (Compositae) exhibited a significant anthelmintic activity when tested in vitro against *Ascaris lumbricoides* and the ethulio coumarin A was found to be responsible for the anthelmintic activity **(Mahmoud *et al*., 1983).** The latex of *Carica papaya* (Caricaceae) was tested for anthemintic activity against natural infection of *Ascaris suum* in pigs

**1.2.3 Plants with antitrematodal effect**

There are many plants have an antitrematodal effect such as *Zingiber officinal* (Zingiberaceae) which commonly known as ginger largely used as carminative and aromatic stimulant. Also it used as anti microbial agent in

the treatment of sores and wounds in traditional medicine and as flavoring agent in beverages **(Adewunmi, *et al*., 1990).** These authors have shown that the pungent phenolic constituents of the rhizome of this plant prevent the egg hatching of *Schistosoma haematobium* the aqueous extract and the rhizomes powdered used to treated the terminal heamatouria in school children who affected by schistosomiasis to reduce the egg count in urine.

## 1.3  Plants used in the present study

### 1.3.1 *Piper abyssinica*

*Piper abyssinica*  refers to the family (Piperaceae), locally known as Show Makkada. It is exist in the eastern and western areas of the Sudan. Many kinds in the genus Piper comprise a number of active constituents including piperdine alKaloid from *P. retrofractum* fruits **(Ahn *et al*., 1992),** phenyl propanoids from *P. cussii* and *P. sumatranum* fruits **(Koul *et al*., 1993),** N-methyl aristolactam, and an oxygenated cyclohexan derivative from *P. ribesioides* fruits and stem **(Ruangrungsi *et, al*., 1992),** 3-4 hydroxy phenyl tetra cosanoate also known as wax ester and betasitosterol from *P. clarkii* leaves and stems **(Boll *et al,* 1992)** and phenolic compounds from *P. nigrum* fruits **(Chiranjib *et al*., 1990).** It has been discovered that eugenol is the important aromatic constituents of the basal oils and the flavour is due to the presence of the anthole in the leaves of *P. bettle* **(Balasubrahmanyam and Rawat, (1992).**

**1.3.1.1 Folkloric uses**

*Piper abyssinica* in sudan is used for the medication of different aliments like dysentery and giardiasis  **(Ali, 1995)**. In west Africa, the fruits of *P. guinecnse* are used as condiments and as constituent of remedial preparation by conventional practitioners **(Abila *et a*l., 1993).** The essential oils in the fruits of *P. longum* (Piperaceae) were noticed to be effective against ascaris

lumbercoides infection, in vitro investigations the piper showed limited effect in the worm preparation if compared to that manufactured by piperazine citrate and tetramisole **(Dwuma - Badu *et al*., 1967; Addae - Mensah *et al*., 1977).**

The insecticidal activity against house flies of *P. guineense* root is due to the presence of methyl terminated a mides pellctorine and akalecide **(Gbewonyo, *et al,* 1993).**

## 1.3.1.2 Toxicity

The seed of *Piper abyssinica* was discovered to be toxic to Nubian goats and chicks. The signs of the toxicity is diarrhoea, bloat, dyspnoea, conjunetivitis, inappetence and recumbency **(Ali 1995).** The aqueous extract of the plant fruit has reverse anticonvulsant effect in mice **(Abila, *et al*., 1993).** Feeding mice with 2mg of a black piper *(P. nigrum)* extract increased the neoplasm bearing mice number **(Shwaireb *et al*., 1991).**

## 1.3.*2 Jatropha curcas* (physic nut) purging nut

*Jatropha curcas* or (physic nut) belongs to the family Euphorbiaceae. The local name is Habet El Muluk, originated in central America and is today found throughout the world in the tropics. In Sudan it found in Southern. and Eastern states The physic nut tree or bush (*Jatropha curcas L.*), with maximum height of five meters, it produces plum size fruit with two  or three oleiferous seeds. The seeds are toxic because they contain curcin (a toxic protein)  **(Broun and Messey, 1929).**

Pure curcin is highly toxic. Therefore seeds, and press cake are unsuitable for human or animal consumption. Because of the toxic and bitter substances, the plants are not eaten by animal. The fruits is an ovid capsule green and fleshy fruits, becoming dry at maturity, brownish or black containing three cavities and two or three black seed.

## 1.3.2.1 Folkloric uses

A cording to **Hart well, 1969** the extracts of *Jatropha curcas* are used in folk remedies for cancer and reported to be anodyne, abortifacient, antiseptic, emetic hemostatic, lactagogue, diuretic, narcotic purgative, rubefficient, styptic, vermifuge, and vulnerary and used for alopecia remedy, anasorca, ascites burns, carbuncles, convulsion, cough, dermatitis, diarrhoea, dysentery, eczema, fever, gonorrhea, hernia, inflammation, jaundices, parlaysis, parturition, pneumonia, rash, rheumatism scabies, sores, stomachache, syphilis, tetanus, thrush, ulcers tumors, neuralgia, and yellow fever **(Duke and Wain, 1981; List** and **Horhammer, 1969)**. Latex applied topically to bee and wasp stings **(Watt and Breyer – Brand Wijk, 1962).** Cameroon native apply the leaf decoction of *Jatropha curcas* in arthritis **(Watt and Breyer – Brand wijk, 1962).** Colombians drink the leaf decoction for venereal disease **(Morton, 1981).** Bahamans drink the decoction for heart burn. Costa Ricans poultice leaves onto erysipelas and spleenosis. Guatemalans place heated leaves on the breast as a lactagoguee. Cubans apply the latex to toothache. Colombians and Costa Ricans apply the latex to burns, hemorrhoids, ring worm and ulcers. Barbadians use the leaf tea for marasmus, Panamanians for jaundic, Venezuelans use the root decoction for dysentery **(Morton, 1981).** Seeds are used also for dropsy, gout, paralysis, and skin ailment **(Watt and Breyer – Brand Wijk, 1962).** Leaves are regarded as antiparastic, applied to scabies; rubefaeient for paralysis. Latex used to dress sores and ulcers and inflamed tongues **(Perry, 1980)**. The seed oil used as emetic, laxative, purgative, and for skin ailments. Root is used in decoction as a mouth wash for bleeding gum and toothache. Homeopathically used for cold sweats, colic, Collapse, cynosis, and diarrhoea..

**1.3.2.2 Chemistry**

100g of the seed of *Jatropha curcas* is reported to contain 6.6g $H_2O$ 18.2g protein, 38.0g fat 33.5.g. carbohydrate, 15,5g fiber, and 4.5g ash **(Duke and Atchley, 1984)**. Leaves, which show antileukemic activity, contain ά amyrin, β- sitosterol, stigma sterol, and campesterol.

Leaves contain isovitexin and vitexin. The nut contain raffinose, sacarose, stachyose, glucose, fructose, galectose, protein and oil, largely of oleic and linoleic acids **(List and Horhammer, 1969 - 1979),** curcasin, arachidic -, linolic, myristic, oleic, palmitic and stearic, acids are also reported **(Perry, 1980).**

### 1.3.2.3 Toxicity

*Jatropha curcas* is poisonous and irritant, with acute abdominal pain and nausea about ½ hour following ingestion. Depression and collapse may occur especially in children. Two seeds are strong purgative. Four to five seed have caused death. Bark, fruit, leaf, root and wood are reported to contain HCN **(Watt and Breyer – Brand Wijk, 1962).** Seeds contain the dangerous toxa albumin curcin.

*J. curcas and J. glauca* cause hemorrhagic gastroenteritis erosions of intestinal mucous membrane, necrosis of the hepatocytes, and epithelial cells of kidney in cattle – sheep and goats **(Kingsbury, 1964; Adam and Magzoub, 1975; Ahmed and Adam, 1979 a; El sayed, 1981).** The seed is toxic to mice, goats sheep, calves and chick **(Adam, 1974; Adam and Magzoub, 1975; Ahmed and Adam, 1979; b, El Badwi, 1990).** The toxicity signs are severe heamorrhages in the stomach, abomasums, and enteritis.

### 1.3.2.4 Description

Shrub or tree to 6m, with spreading branches and stubby twigs with yellowish rufescent exudates, Leaves deciduous, alternate but apically crowded, ovate acute to acuminate, basally cordate, 3 to 5 lobed in outline, 6-40cm long, 6-35 cm broad, the petioles 2.5-7.5 cm long.

Flowers several to many in greenish cymes, yellowish, bell shaped sepals 5, broadly detoid. Male flowers many with 10 stamens, 5 united at the base only, 5 united into a column, female flowers borne singly, with elliptic 3 celled, triovulate ovary with 3 spreading bifurcate stigmat a. Capsules, 2.5 – 4 cm long, finally drying and splitting into 3 valves, all or two of which commonly have and oblong black seed, these ca 2 x 1 cm **(Morton, 1977; little *et al.,* 1974).**

**1.4 Drugs used as a reference**

## 1.4.1 A vermectin

The names Abamectin and Ivermectin describe the same class of compounds comprising Avermectin Bla, Avermectin Bib, Ivermectin, and Abamectin. They are all Called Avermectin here for simplicity. Avermectins are insecticidal compounds extracted from the soil bacterium *streptomyces avermitilis* originally explored in Japan. Avermectin is a ntural fermentation product of this bacterium (U.S) Environment protection agency (EPA) classified the compound as class IV toxicity or practically nontoxic and fully evaluated by EPA for its effects on human health or the environments. Presently, avermectins are the active components of some insecticidal and nematocidal products.

**1.4.2 Uses and mode of action**

The compounds act as an insecticide by interfering with the nervous system of the insect and this lead to the paralysis of insect. Avermectin is

used to control insect and mite pests of ornamental plants in green houses, such as spider mites it is formulated into an enclosed capsule system for tree injections it is a very common veterinarian medicine for treatment of internal and external parasite and mites of pets and livestock, including scabies. It is formulated into several commercial baits for cockroaches (Avert brand cockroach bait) and ants, including an effective formulation for the control of carpenter ants (advance brand carpenter and bait). Ivermactin is effective to treat human onchocerchiasis (River blindness) has made it a promising candidate for the control of one of the most insidious and intercatable tropical disease **(Campbell, 1989). Smith and Campbell 1996),** showed that the temporary paralysis, including suppression of pharyngeal pumping, does not protect L1 larvae of *C. elegans* from the effect of Ivermectin. This with the capacity of Ivermectin to stop pharyngeal pumin adult nematodes at concentrations much lower than those required for whole body paralysis. **Geary *et al*. (1993),** suggest that the drug may enter the nematode body by crossing the cuticle or by entering opening other than the mouth. Recent studies on the transport kinetics of anthlmetics when applied to parasitic nematodes in vitro suggest that transcticuler absorption may be common mode of entry for antinematodal drugs **(Ho *et al* 1994).**

## 1.4.3 Toxicity

Avermectin is highly toxic to insects and mites; however it has very low mammalian toxicity when tested on rats, toxic doses is more than 5 gram/kilogram of body weight were required to kill 50% of the tested animals (LD50). Emulsifiable concentrate formulations used in green houses may cause slight to moderate eye irritation and mild skin irritations. At very high doses, it can affect mammals, causing symptoms of nervous system depression such as incoordination tremors, lethargy, excitation, and pupil

dilation. It is not readily absorbed through skin. Tests with monkeys show that less than 1% of dermally applied abamactin was absorbed into the blood stream through the skin.


### 1.4.4 Pharmacokinetics of avermectin in goats

Pharmacokinetics and mammary excretion of ivermectin were confirmed in goats following subcutaneous administration. Kinetic analysis of plasma and milk levels was performed using a one – compartment model. The maximum plasma concentration of 6.12 mg/ml occurred at 2.85 days. The half life of 4.03 days was similar to the value in sheep (3.68 days). Ivermactin was detected in the milk at the first sampling and there after for at least 25 days **(AL vinerie *et al*, 1993).**

Bioavailability was lower with percutaneous administration. Oral administration gave peak plasma concentration (15.85mg/ml) within one day and clearance by 72-120 hours. Percuaneous administration gave peak plasma concentration (about 3.8mg/ml) within two days and clearance by 192 hours. Ivermectin concentration in milk was lower than in plasma **(Scott *et al*., 1990).**

**1.4.5 Uses of avermectin in goats**

Ivomec or Oramec liquid for goats is the same 0.08% w/v solution of Ivermectin as used in sheep at dose 2.5ml/10kg body weight. The product is indicated for treatment and control of gastrointestinal nematodes and lung worms in goats, there is insufficient efficacy against nematodes to justify use of the injectable formulation (Ivomecin jection) **(Benz *et al*., 1989).**

CHAPTER TWO

## MATERILS AND METHODS

## 2.1 Materials and experimental designs

*2.1.1 Anthelmintic activity of* piper abyssinica *against* Haemonchus contortus *infection in goats.*

## 2.1.1.1 Experimental animals

Twenty 7 month old Desert male goats weighing 10.2kg to 13.1kg were purchased from local livestock market at Nyala town. They were ear-tagged and housed in clean pens in the permesis of the Regional Veterinary Research Labrotary. They were initially given prophylactic anthlemintic treatment (Albendazole, 2.5% at dose rate of 1ml/10kg b.wt. orally), and vaccinated against PPR, HS and goat pox. They were allowed an adaptation period of three weeks before the experiment commenced. The animal were all subjected to through clinical examination and blood and faecal samples were further examined to insure that the animal are healthy and clean from internal parasites. The animals were housed in four separated pens with free access to food (groundnut hay) and fresh drinking water was available *ad libidum*.

## 2.1.1.2 Infection and treatment

The experimental animals were divided into four equal groups A, B, C and D according to their body weight, 5 animals in each group. The animals were orally administrated with the larval suspension at a dose rate of 125 larvae/kg body weight. All the groups were infected with $L_3$ (stages three) of *Haemonchs contortus*. The Blood collection for measurements of Hb, PCV and RBC count were done. The measurement of body weight was done initially and after treatment commenced. *Piper abyssinica* was given to group A at dose 500mg/Kgbw, group B at dose 1000mg/kgbw, group C was treated with avermectin from soil bacterium *streptomyces avermitilis* at

dose rate 200μ/kgbw and group D was left as infected untreated control. These doses were repeated again at day 21 post infection, except for group C was treated with single dose Ivermectin. All the groups were slaughtered at day 25 post infection (day 6 of treatment)

Details of experimental designs for treatment with *P.abyssinca* are given in Table (2).

**Table (2) Treatment of experimental groups with *P. abyssinica***

| Groups | average b.weight (kg) | Treatment | Dose mg/kg | Route of administration | Day of treatment pi |
|--------|------------------------|-----------|------------|--------------------------|----------------------|
| A | 11.8 | P.abyssinica | 500 | Oral | 20 & 21 |
| B | 13.1 | P.abyssinica | 1000 | Oral | 20 & 21 |
| C | 12.1 | Avermectin | 200μg/kg | Subcutaneous | 20 |
| D | 10.2 | Control | D.water | oral | -- |

Pi = post infection

**2.1.2 Anthelmintic activity of *Jatropha curcas* against *Haemonchus contortus* infection in goats.**

**2.1.2.1 Experimental animals**

Twenty - seven month old Desert male goats weighing 9.8kg to 12.1kg were purchased from local live stock market at Nyala town, they were ear tagged and housed in clean pens in the premises of the Regional Veterinary Research laboratory, they were initially given porphylactic anthlimntic treatment (Albendazole, 2.5%) at dose rate of Iml/10 kg b. wt. orally and vaccinated against PPR, HS and goat pox.

They were Kept for three weeks before the experiment started as adaptation period.   The animals were all subjected to through clinical examination and blood and faecal samples were further examined to insure that they are healthy and clean from internal parasites.

The animals were housed in four separated pens with free access to food (ground nut hay) and fresh drinking water was available *ad libidun*

## 2.1.2.2    Infection and treatment

The experimental animals were divided into four equal groups according to their body weight, 5 animal in each group. The animals were orally inoculated with the larval suspension at dose rate of (125) larvae/kg body weight. All the groups were infected with L3, third stage larvae, of *Heamonchus contortus*. The Blood collection for measurement of Hb, PCV, RBC count were done and the measurement of body weight was done initially and at the end of the treatment. *Jatropha curcas*  was given to infected groups A at dose (250mg/kg /b. w) group B at dose (500 mg/ kg bw), group C treated with Ivermactin at dose rate (200 µ/kg) and group D was left as infected untreated control (Table 3). The doses had been repeated again at day 21 post infection except group C (treated with the Ivermectine one dose only) all the groups had been slaughtered at day 25 post infection (day 6 of treatment).

**Table (3) Treatment of infected groups with *J. curcas***

| Groups | average weight (kg) | Treatment | Dose (mg/kg) | Route of administration | Day of treatment Pi |
|---|---|---|---|---|---|
| A | 10 | **J.curcas** | 250 | Oral | 20 & 21 |

| | | | | | |
|---|---|---|---|---|---|
| B | 9.8 | **J.curcas** | 500 | Oral | 20 & 21 |
| C | 12.1 | Avermectin | 200µg/kg | Subcutaneous | 20 |
| D | 10.2 | Control | D. water | oral | -- |

Pi = post infection

### 2.1.3 Parameters

Blood samples were collected from the jugular vein at weekly intervals before and after infection of experimental animals. A small volume of the blood was withdrawn in a vacutainer tube containing EDTA for hematological investigation. The rest of the blood was collected in clean vials without anticoagulant for serum separated. The serum was immediately stored at $-20^0$C till analyzed.

## 2. Methods

## 2.1 Plant materials

### 2.1.1 Piper abyssinica

The seeds of *Piper abyssinica* was bought from the market at Nyala town. and was been as then crushed with a pestle in morter and made into powder form. The dose was measured according to the live weight of the infected animal. The powder was suspnded in water and drenched to the animals orally using a bottle at day 20 and 21.

### 2.1.2 Jatropha Curcas

The seeds of *Jatropha Curcas* was bought from the market at Nyala town. and was been as then crushed with a pestle in morter and made into powder form. The dose was measured according to the live weight of the infected animal. The powder was suspnded in water and drenched to the animals orally using a bottle at day 20 and 21.

### 2.2 Parasitological Methods

## 2.2.1 Collection of abomasal samples worms

A total of 50 abomasi were collected from the local abattoir in Nyala town. Each abomasum was ligated from both ends and immediately sparated from the rest of the digestive tract. The abomasi were placed in a large plastic bowl and each one was opened along its greater curvature.

The abomasal contents were poured in a large plastic bowl and then the abomasal mucosa was gently washed with running tap water. The attached worms on the mucosal surface were removed and the content of the bowl were sieved through a wire mesh screen (0.15mm) aperture. The material caught by the sieve was continuously washed with flow of tap water until no colored material of food particles were passed through the sieve. The contents were then inverted into another bowl and its content was furtherly washed by running water into the bowl. The bowl content was then transferred in small amounts into a petri dish and examined for adult *Haemonchus contortus*.

Isolation of pure *Haemonchus contrtus* egg and mature gravid Haemonchus females were collected directly from the contents of abomasi of naturally infected goats. All the worms were put in a mortor and purified sand was added and then crushed with pestle to expel the eggs out of uteri of the worms.

## 2.2.2 Egg culture

The feacal culture was prepared according to **Urquhart *et al.* (1996).** Sterilized egg-free horse faeces were crushed into small particles, a small amount of water was added to the faecal mass to form a moist crumbly material. The pure eggs were then added, and well mixed, the faecal mass

was then wraped into a piece of gauze and suspended to the cover of a closed marmalade jar containing water at the bottom to provide moisture. The jar was then incubated in a dark place at room temperature for 12 days. Harvesting of *Haemonchus contortus* infective larvae was done by application of Baermann Technique described by **Urquhart *et al.* (1996)**. The wraped gauze was placed in a closed funnel (Baermann apparatus) containing warm water and allowed to stand for three hours. The infective larvae then move down by their own activity from the feacal material to the stem of the funnel. The larval suspension was carefully collected into clean glass containers.

### 2.2.3 Infection

one ml of the larval suspension was pipetted onto a glass slide with a cover slip for microscopic examination and counting under low magnification. The number of infective larvae per ml were given as a mean of five repeated readings. The amount of larval suspension (ml) required for infection of experimental animals was determined as follows:

larval suspension (ml) = Body weight. (kg) x infective dose (larvae / kg body weight)

The number of larvae /ml

The experimental animals were thoroughly observed for clinical changes with especial attention been given to appetite, general body condition, visible mucous membranes and consistency of faeces. The body weight for each experimental animal was determined at weekly intervals throughout the experimental period.

Faecal samples were collected daily from the rectum of the experimental animals and kept in plastic containers for immediate examination and egg count.

## 2.2.4 Total worm burden

Total worm counts included adult and immature worms. This was done according to the methods described by **Urquhart et al. (1996)** The abomasi were placed on large plastic bowl and each one was open along It's greater curvature. The abomasal contents were poured on a large plastic bowl and then the obomasal mucosa was gently washed with running tab water. The attached worms on the mucosa surface were then removed by fingers. The content of the bowl were then sieved through a wire mesh screen (0.15mm) aperture. The material caught by the sieve was continuously washed with a low flow of tap water until no colored material of food particles were passed through the sieve. The content was then inverted into another bowl, and its content was further washed by running water into the bowl, a small amount of the bowl content was then transferred into a petri dish, and then examined. Adult *Haemonchus contortus* worms were identified accourding to morphological criteria stated by **Urquhart et al. (1996)** The collected worms were removed into another dish containing 10% formalin and were individually counted. The numbers of the male and female worms were also determined. The immature worms were furtherly recovered by digestion of the abomasal mucosa in pepsin / HCl (8g pepsin, 20 ml concentrated HCl + 23ml saturated NaCl in 940 ml $H_2^o$ ). The whole abomasal mucosa was scraped off and removed into flask containing the digestive fluid (100-150ml). The flask with it's content was then placed on a magnetic sterier adjusted to 5rpm for three hours until the mucosal tissue was completely digested. The digested material was poured through two sieves (0.0 75 and 0.038 mm aperture). The material remaining on the sieves was then washed with ejects of tab water. The sieves content was washed in ajar and the volume was completed to 500 ml with tab water. l0ml of the fluid (after well

mixing) was taken in a test tube, then few drops were pipetted into a glass slide with a cover slip for microscopic examination and counting at low magnification.The immature larvae burden per animal was determined by multiplying the number of larvae sampled from the I0ml fluid by 50.

### 2.2.5 Faecal egg count

Feacal egg count was perfomed by the use of a modified Mc Master technique **(Urquhart *et al.,* 1996).** Three grams of faeces were throughly homogenized and mixed with 42 ml of tab water in a wide mouth stoppered bottle. The faecal suspension was well mixed and was then filtrated through a wire mesh screen (0.15 mm aperture), fifteen ml of the filtrate was furtherly centrinfuged for two minutes at 1500 rpm. The supernatant was then discarded and the packed sediment was emulsified with saturated sodium chloride (NaCl) solution up to the pervious volume of initial liquid (15ml). Two Mc Master champers were filled with the tube content using a clean Pasteur pipette and examined under the microscope (low magnification). *Heamonchus contortus* eggs were then counted within the entire marked areas. The number of eggs per gram of faeces (EPG) were calculated by multiplying the number of eggs (counted in the both champers) by fifty.

### 2.3 Patholgical methods

### 2.3.1 Post mortem examination

Post mortem examination was performed at the end of treatment. The carcass and all organs were thoroughly examined for gross lesions with special attention been given to the gastrointestinal tract. In addition, the abomasi were ligated, removed and carefully examined for the presence of worm and related lesions. All mature and immature worm were recovered and counted as previously described.

Specimens of the liver, spleen, kidney, lung, abomasum and intestine were immediately collected and fixed in 10% buffered formal saline.

## 2.3.2 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 preformed by rotatroy Microtome (Baired and Totlock, England) and the section (5- 6 μm) were fixed in glass slides and allowed to dry.

They were then freed from water, rehydrated, cleared and finally stained with

heamatoxylin and eosin (H&E). The stained sections were covered with slips, fixed with

Canada balsam and allowed to dry for 24hours before being examined.

## 2.4 Biochemical methods

## 2.4.1 Total serum protein

Total serum protein concentration was determined by the Biuret method using commercial kits (Fouz Diagnostics Laboratory, Sudan) the principal of the method is based on the reaction of protein with copper sulphate in the presence of Sodium hydroxide (NaOH). Protein in serum forms a blue violet complex when mixed with copper ions alkaline solution (Biuret reaction), each copper ion binding with 5 or 6 peptide bonds. Tartarate is added as stabilizer and iodine is used to prevent auto reduction of the alkaline copper complex. The absorbance of this complex at 546nm is proportional to the protein concentration. The optical density of the developing color was measured at 540nm using Corning colorimeter, 252, U.K. The total serum protein value was measured in gm/dl as follows:

Total serum protein concentration = $\underline{\Delta \text{ Asample}}$ x concentration of standard

Δ A standard

## 2.4.2 Serum albumin

Serum albumin concentration was determined by the bromocersol green (BGG) methods described by **Northon and Widdowson (1967).** The specific binding of the dye (BGG) with albumin result test in a changed peak absorbance wavelength. The serum samples and standard were added to the buffered solution of BCG (Sodium nitrite buffered, pH 3.7) and incubated for 10 minutes at 20$^o$C. Changes in the absorbance were recorded at 540 nm against a working buffered BCG blank. The result was calculated following the formula:

Albumin concentration (g /dl) = Absorbance of sample x standard concentration

Absorbance of standard

## 2.4.3 Serum urea

Serum urea concentration was measured by an enzymatic colorimetric method using commercial kits (Fouz Diagnostics Laboratory, Sudan).

Urea in the presence of water and urease was hydrolyzed to produce ammonia and carbon dioxide. In a modified Berthelot reaction, ammonia ions react with hydrochloride and salicylate to give a green dye. The increase of absorbance at 578nm is proportional to urea concentration in the sample.

The absorbance of the sample and standard were read against blank in the colorimeter at 570 nm and the concentration of serum urea was calculated as follows:

Urea concentration (mg/dl) = Δ Asample   x concentration of standard

Δ Asandard

## 2.4.4 Serum creatinine

Serum creatinine concentration was measured by an enzymatic colorimetric method using commercial kits (Fouz Diagnostics Laboratory, Sudan.

Creatinine in alkaline solution reactsd with picric acid to form a colored complex. The amount of the complex formed was directly proportional to the creatinine concentration. After 30 seconds from sample preparation, absorbances of sample and standard were read. Exactly two minutes later absorbances of sample and standard were read again.

Calculation:

Creatinine concentration (mg/dl) = $\underline{\Delta \text{ Asample}}$   x concentration of standard

$$\Delta \text{ Asample}$$

## 2.4.5  Serum bilirubin

Serum bilirubin (total and direct bilirubin) concentration was measured by **Jendrassik – Grof (1938)** method using commercial kits (Fouz Diagnostics Laboratory, Sudan). Bilirubin react with diazotized sulphanilic acid (DSA) to form a red azo dye. The intensity (at 546nm) is directly proportional to the bilirubin concentration in the sample. Water soluble bilirubin glucoronides react directly with DSA whereas the indirect bilirubin will only react with DSA in presence of an a accelerator

Total − direct = indirect bilrubin.

Sulphanilic acid + sodium nitrite → DSA

Bilirubin + DSA  direct → Azo bilirubin

Bilirubin + DSA+ accelearator→ total Azo bilirubin

 Bilirubin concentration = AS x 13.2 (mg/dl)

## 2.4.6 Serum total iron concentration

Serum iron concentration was measured by iron ferrozine colorimetric method endpoint using Linear Chemical- SL, Spain, commercial kits . The $Fe^{+++}$ bonds to serum ferritin once dissociated in a weak acid medium by

teepol and guandium chloride, was reduced by hydroxylamine to $Fe^{++}$ forming the ferrous ion colored complex with ferrozine.

The concentration of ion present in the sample was calculated as fallows: iron concentration (ug/dl iron). = $\dfrac{\text{absorbance of sample - absorbance blank}}{\text{Absorbance of standard}}$ x concentration of standard

## 2.5 Haematological methods

### 2.5.1 Haemoglobin concentrations

Hemoglobin concentration (Hb) was determined by the acid haematin method **(kelly, 1984)** using hemoglobinometer. The hemoglobin concentration was measured in g/dl of blood by the following formula:

Hemoglobin concentration (g/dl) = $\dfrac{\text{Hb\% x14}}{100}$

### 2.5.2 packed cell volume (PCV)

Blood samples were drawn into heperinized capillary tubes sealed at one end with critaseal (Hawksley and Sons Ltd, Engleand), and centrifuged for five minutes using microhaematocrite centrifuge (Hawksley and Sons Ltd, England). The PCV% was read in Hawksley microhaematocrit reader.

### 2.5.3 Red blood cell count (RBC)

Erythrocytes were counted with an improved Neubaur Haemocytometer (Hawksley and Sons Ltd, England). Formal citrate was used as a diluent.

### 2.6  Statistical analysis

Student's t- test was used in the present study to compare mean values in body weights, worm counts, blood and serum according to **Mendanhall (1971).**

# CHAPTER THREE

# RESULTS

## 3.1 Effect of powdered seed of *Piper abyssinica seed* against experimental *Haemonchus contortus* infection in goats.

### 3.1.1 Efficacy

The efficacy of powdered seed of *Piper abyssinica* seed against *Haemonchus contrutus* infection in goats compared with Ivermectin is summarized in Table (4).

**Table (4) Efficacy of powdered seed of *Piper abyssinica* against experimental *Haemonchus contortus* infection in goats**

| Group | No. of animals | No. of larvae administered | Dose | Mean abomasal (worm) | Efficacy (%) |
|-------|----------------|----------------------------|------|----------------------|--------------|
| A | 5 | 125 | 500 mg/kg *P.abyssinica* | 388 | 18.65 |
| B | 5 | 125 | 1000mg/kg *P.abyssinica* | 203 | 57.40 |
| C | 5 | 125 | 200 µg/kg Ivermectin | 2 | 99.55 |
| D | 5 | 125 | Untreated control | 477 | 000 |

All treated groups showed reduction in worm numbers when compared with the control group. Group A which was treated with 500mg *P. abyssinica* showed 18,6% efficacy when compared with untreated control. Group C which was treated with Ivermectin showed 99,5% efficacy. Group B receiving 1000mg/kg. *P. abyssinica,* showed 57.4% efficacy when the compared with untreated control.

### 3.1.2 Number of egg per gram of feces (EPG):

Count of *Haemonchus controtus* eggs per gram of feces in goats treated whith *Piper abyssinica* is summarized in Table (5).

**Table (5) Count of *Haemonchus contortus* eggs per gram of feces in goats treated with *Piper abyssinica***

| Group | Dose | days | | | | |
|-------|------|------|------|------|------|------|
| | | Infection | | Treatment | | |
| | | 17 | 19 | 2 | 3 | 6 |
| A | 500 mg/kg *P.abyssinica* | 520±10*** | 6.233±30 N.s | 5.183±80*** | 4.116±60*** | 3,080±3*** |
| B | 1000 mg/kg *P.abyssinica* | 340±10 N.S | 4.660±50 N.S | 2.480±10*** | 4.220±10*** | 2.880±2*** |
| C | 200/µg/kg Ivermectin | 1.020±5*** | 1.760±10*** | 1.100±50*** | 120±10*** | 0 |
| D | Untreated Control | 360±10 | 2.880±10 | 6.800±10 | 8.620±10 | 13.560±10 |

P<0.001=*** NS=Not significant

*Haemonchus contortus* eggs started to appear in faeces at day 17 post infection. The maximum egg shedding was show in group A (500mg/kg) and group B (1000mg/kg) *P.abyssinca* and group C which was treated with Ivermectin at day 19. Post infection. There was significant decrease in egg shedding in all treated groups post treatment. There was significant reduction in egg count (P<0.01-0.001) from the second day of treatment till day 6 post treatment in the treated group and this reduction is proportional to the dose. The egg shedding in group C that was treated with Ivermectin was significantly decrease until disappeared at day 6 post treatment.

### 3.1.3 Body weight

The results of body weight changes in goats treated with *Piper abyssinica* at doses 500 and 1000 mg/kg are presented inTable (6)

**Table (6) Average body weight (kg) in goats treated with *Piper abyssinica***

| Group | Dose | Initial weight(kg) | Final weight (kg) |
|-------|------|--------------------|--------------------|
| A | 500mg/kg *P.abyssinica* | 14.8±0.32 N.S | 10.7±0.11*** |
| B | 1000mg/kg *P.abyssinica* | 13.14±0.43 N.S | 10.5±0.12*** |
| C | 200/µg/kg Ivermectin | 12.1±0.1 N.S | 10.4±0.11*** |
| D | Untreated Control | 13.9±0.31 | 7.6±0.10 |

All treated groups showed reduction in their final body weight when compared to the initial body weight. This reduction is more severe in untreated control and it is significantly different (P<0.01-0.001) in the treated groups when compared to untreated control (Fig.1).

## 3.14. Clinical Signs

Goats infected with *H.contortus* at day 7, los their appetite and became dull, depressed and they further became  weak and emaciated with rough coat (Fig.2). The visible mucous membranes were pale.  Goats in group C treated with Ivermectin showed gradual improvement in their appetite and general condition and appeared healthy, and those treated with *P.abyssinica* also showed improvement in their condition but less than those treated with Ivermectin.

**3.1.5 Post mortem Findings**

In all infected groups the carcass was pale emaciated and with reduced muscular mass with gelatinization of body fat (Fig. 3). The abomasi contained a small amount of dark brown fluid. The mucosa of abomasi were pink in colour, the abomasal folds were thin and oedematous, visible numbers of adult worm were found within the abomasal content or closely adherent to the a bomasal mucosa. In group A (500 mg/kg) and group B (1000mg/kg) *P. abyssinica* there were petecheal haemorrhages in the liver and kidneys and renal blood vessels were congested. In group C treated with Ivermectin there was slight congestion in the kidneys.

**3.1.6 Histopathology**

In goats treated with *Piper abyssinica* at 500 mg/kg there were slight congestion in the liver and small intestine. The spleen showed congestion and deposition of

haemosiderin (Fig.4). In group B (1000mg/kg) there were slight congestion in the kidney (Fig.5) and sever congestion and haemorrhage in abomusum and there was generalized fatty change  in the liver (Fig. 6). The intestine showed congestion of intestinal bood vessels. In groups C treated with Ivermectin, the liver and small intestine showed slight congestion and fatty change and the abomasi and spleen showed congestion and haemorrhage. There were no pathological changes in the kidneys.  In group D (untreated control) there was severe congestion in the abomasi and infiltration of inflammatory cells in the mucosa. The spleen showed severe haemorrhage and haemosidrosis.

### 3.1.7 Haematological findings

Table 7 shows the Hematological changes in the blood of goats treated with *P.abyssinica*

**Table (7): Hematological changes in blood of goats treated with *P.abyssinica*.**

| Group | Dose | Hb (g/dl) | PCV (%) | RBC 6 (x 10) |
|-------|------|-----------|---------|--------------|
| A | 500mg/kg P.abyssinica | 6.7±0.10*** | 25.1±0.12*** | 4.18±0.12*** |
| B | 1000mg/kg P.abyssinica | 5.9±0.15*** | 28.4±0.11*** | 4.7±0.12*** |
| C | 200 µg/kg Ivermectin | 6.9±0.12*** | 32.1±13*** | 5.35±0.13*** |
| D | Untreated control | 4.3±0.01 | 15.8±0.12 | 2.6±0.1 |

P<0.001=***          NS = Not significant

The Hb concentration was significantly increased in the treated groups when compared to untreated control and this increase is more clear in Ivermectin group. In

PCV percentage there was significant increase (P<0.01-0.001) in treated groups when compared to the untreated control.

The red blood cells were increased in the treated group and this increase is proportional to the dose when compared to untreated control, this increase is more clear in group C which was treated with Ivermectin.

### 3.1.8 Biochemical findings

Change in biochemical values in goats treated with *Piper abyssinica* are illustrated in Table (8)

**Table (8) Biochemical changes in goats treated with *P.abyssinica* seed against experimental *H.contortus* infection**

| Group | Dose | Total protein g/dl | Albumin (g/dl) | Urea mg/dl | Creatinine mg/dl | Bilirubin mg/dl | Iron µg/dl |
|---|---|---|---|---|---|---|---|
| A | 500mg/ke *P.abyssinica* | 7.59±0.2*** | 3.55±0.02*** | 13.35±1.01*** | 0.6±0.1 NS | 0.37±0.01*** | 213±3.00*** |
| B | 1000mg/kg *P.abyssinica* | 8.05±0.01*** | 3.1±0.10*** | 33.3±1.00 N.S | 0.46±0.01** | 0.70±0.1*** | 200±2.00*** |
| C | 200µg/kg *I.vermectin* | 7.08±0.01** | 3.3±0.10*** | 37.6±0.10 N.S | 0.6±0.1 N.S | 0.50±0.01*** | 233±1.00*** |
| D | Untreated control | 6.48±0.01 | 2.75±0.01 | 29.9±2.31 | 0.6±0.01 | 1.1±0.1 | 100±2.00 |

P<0.01=**          P<0.001=***          N.S= Not significant

Total protein was significantly increased in all treated groups (P<0.01-0.001) when compared with untreated control. All treated groups showed significant increase in albumin concentration (P<0.001) when compared with the untreated control group. Serum urea concentration was significantly decreased in group (A) which was treated with 500mg/kg *P. abyssinca* when compared with the control group.

The other treated groups showed no significant changes in serum urea concentration when compared with the untreated control. There was no significant difference in serum creatinine concentration in group A which was treated with 500mg/kg *P. abyssinca* and Ivermectin group when compared with the untreated control whereas group B which was treated with 1000mg/kg *P.abyssinica* showed significant decreased in serum creatinine concentration when compared with the untreated control. Serum bilirubin concentration was significantly decrease in all treated groups (P<0.01-0.001) when compared with untreated control.

Serum Iron concentration was significantly increase in all treated groups (P<0.01-0.001) when compared with untreated control. This increase is directly proportional to the dose.

**3.2 Effect of powdered seed of *Jatropha curcas* against expermintal *Haemonchus contortus* infection in goats.**

3.2.1 Efficacy of powdered seed of *Jatropha curcas* against *Haemonchus contortus* infection in goats compared with Ivermectin are summarize in Table 9

**Table (9) Effect of powdered seed of *Jatropha curcas* against expermintal *Haemonchus contortus* infection in goats.**

| Group | No. of animals | No. of larvae administered | Dose | mean abomasal (worm) | Efficacy (%) |
|-------|----------------|----------------------------|------|----------------------|--------------|
| A | 5 | 125 | 250mg/kg J.curcas | 438 | 8.10 |
| B | 5 | 125 | 500mg/kg J. curcas | 270 | 43.30 |
| C | 5 | 125 | 200 µg/kg Ivermectin | 2 | 99.55 |
| D | 5 | 125 | Unreated control | 477 | - |

All treated groups showed reduction in worm numbers when compared with control group. Group A which treated with 250mg/kg *Jatropha curcas* showed (8.1%) efficacy when compared with untreated control. Group C which treated with Ivermectin showed (99.55%) efficacy. Group B receiving 500mg/kg *J.curcas* showed  (43.3%) efficacy when compared with untreated control.

**3.2.2 Number of egg per gram of feces  (EPG)**

The count of *Haemonchus contortus eggs* per gram of faces in goats treated with *Jatropha curcas* was summarized in Table (10)

**Table (10) count of *Haemonchus contortus eggs* per gram of faeces in gaots treated with *Jatropha curcas* and Ivermection**

| Group | Dose | days | | | | |
|-------|------|------|------|------|------|------|
| | | Infection | | Treatment | | |
| | | 17 | 19 | 2 | 3 | 6 |
| A | 250mg/kg J.carcus | 640±17** | 2.280±10*** | 4200±5*** | 5.700±1 5*** | 805±10*** |
| B | 500mg/kg J.carcus | 2000±5** | 3.600±5*** | 4.220±20*** | 420±20*** | 420±1*** |
| C | 200µg/kg Ivermectin | 1.020 ±5** | 1.760±10*** | 1.100 ±5*** | 120±1*** | 0 |
| D | Control | 960±10 | 2.880±10 | 6.800±10 | 8.620±10 | 13..560±10 |

P<0.001=***          N.S = Not significant

There was significant difference in egg shedding in all infected groups. The maximum egg shedding was occurred at day 19 post infection. In all treated group there was significant decrease in egg shedding was observed at day 6 post treatment in all treated group when compared with untreated control. In Ivermectin group the egg shedding was significantly decrease until disappear at day 6 post treatment. The decrease in treated groups was dose correlated.

**3.2.3 Body weight**

The changes of body weight in goats treated with *Jatropha curcas* are illustrated in Table (11)

**Table (11) Average body weight (kg) in goats treated with *Jatropha curcas*.**

| Group | Dose | Initial weight (kg) | Final weight (kg) |
|-------|------|---------------------|-------------------|
| A | 250mg/kg *J.curcas* | 12±0.32 N.S | 10.8±0.13*** |
| B | 500mg/kg *J.curcas* | 11.84±0.23 N.S | 9.7±0.14*** |
| C | 200/µg/kg Ivermectin | 12.1±0.21 N.S | 10.4±0.11*** |
| D | untreated Control | 11.4±0.21 | 7.6±0.12 |

P<0.001=***          N.S = Not significant

All treated groups showed reduction in their body weight and this reduction is more severe in untreated control group. And it is significantly different in the treated groups when compared with untreated control, but still the body weights in treated groups is better than that in untreated control. (Fig. 7)

**3.2.4 Clinical signs**

Goats infected with *H.contotrus* at day 7, showed depressed appetite and dullness, weekness and emaciation with pale visible mucous membrane and rough coat. All goats treated with *J.curcas* at dose 250mg/kg 500mg/ show diarrhoea at day 6 post treatment where as the goats treated with Ivermectin showed gradual improvement in their appetite and general condition and appeared healthy.

### 3.2.5 Post mortem

In all infected groups, the carcass was pale emaciated and there was gelatinization of body fat. The mucosa of abomasum was pink in colour. The abomasal folds were thin and oedematous, variable numbers of adult worm were found within the abomasal content or closely adherent to the abomasal mucosa. In group A (250mg/kg) and group B (500mg/kg) the liver was pale, congested and fatty change was seen. The kidney was pale and showed slight haemorrhage, the abomasi was congested and inflamed. In small intestine there was inflammatory changes. In control group the liver was pale and fragile. In Ivermetin group there was petechial haemorrhage in the kidney.

**3.2.6 Histopathology**

In group A which treated with 250 mg/kg *J.curcas* there were slight congestion in the kidney and small intestine and congestion in abomasal mucosa and inflammation of the gastric glands, kidneys showed lymphocytes infiltration. In group B which treated with 500mg/kg *J.curcas* there was slight congestion and fatty change in the liver (Fig. 8) and kidney showed  slight congestion (Fig. 9) and hemorrhage in the abomasi, the spleen showed haemosdrosis. There was no change in the small intestine. In group C treated with Ivermectin, kidenys and small intestine showed slight congestion and fatty change and liver showed fatty changes, while the abomsmi and spleen showed congestion and hemorrhage.  In control group D there was severe congestion in the abomasi and infiltration of inflammatory cell in the muosa of intestine and spleen showed sever haemorrhage and haemosidrosis.

### 3.2.7 Haematological finding

Table (12) describes hematological changes in blood of goats treated with *J.curcas*

**Table (12) Hematological changes in blood of goats treated with *J.curcas***

| Group | Dose | Hb (g/dl) | PCV (%) | RBC 6 (x 10) |
|-------|------|-----------|---------|--------------|
| A | 250mg/kg *J.curcas* | 4.96±0.21** | 20.25±0.11*** | 3.38±0.21* |
| B | 500mg/kg *J.curcas* | 4.80±0.15 N.S | 24.25±0.13*** | 4.04±0. 13*** |
| C | 200 µg/kg Ivermcatin | 6.9±0.10*** | 32.1±1.00*** | 5.35±0.11*** |
| D | Untreated control | 4.3±0.01 | 15.8±0.10 | 2.6±0.14 |

P<0.05=*,          P<0.01=**,      P<0.001=***, N.S=Not significant

The Hb concentration was significantly increase in group A which treated with 250 mg/kg *J.curcas* and Ivermectin group (P<0.01-0.001) this increase is more clear in Ivermectin group. In PCV values there where significant increase (P<0.01-0.001) in all treated group when compared to untreated control. The red blood cells was increased in all treated group and this increase is proportional to the dose when compared to untreated control. This increase in more clear in group "C" which treated with Ivermectin.

**3.2.8 Biochemical findings**

Table (13) show biochemical change in goats treated with *J.curcas* against experimental *H.contortus* infection.

**Table (13) Biochemical changes in goats treated with *J.curcas* seed against experimental *H.contortus* infection**

| Group | Dose | Total protein (g/dl) | Albumin (g/dl) | Urea (mg/dl) | Creatinine (mg/dl) | Bilirubin (mg/dl) | Iron (µg/dl) |
|---|---|---|---|---|---|---|---|
| A | 250mg/kg *J.curcas* | 6.95±0.2* | 3.08±0.01*** | 47.1±2.12*** | 0.4±0.01*** | 1.48±0.01*** | 266±6*** |

| Group | | | | | | | |
|---|---|---|---|---|---|---|---|
| B | 500mg/kg *J.curcas* | 7.05±0.01*** | 3.41±0.1*** | 37.6±1.01* | 0.4±0.01*** | 1 ±0.1 N.S | 300±1.00*** |
| C | 200µg/kg I.vermectin | 7.08±0.01*** | 3.3±0.1*** | 37.6±0.1 N.S | 0.6±0.1 N.S | 0.50±0.01*** | 233. ±1.00*** |
| D | Untreated control | 6.48±0.01 | 2.75±0.01 | 29.9±2.31 | 0.6±0.01 | 1.1±0.1 | 100±2.00 |

$P<0.05$=*,  $P<0.00$=***,  N.S = Not significant

Total protein was significantly increase in all treated group ($P<0.01$-0.001) when compared with untreated control. All treated groups showed significant increase in serum albumin concentration ($P<0.001$) when compared with untreated control group. There was no change in serum urea concentration in Ivermectin treated group and significantly increase in groups treated with *J.curcas* at dose (250mg/kg and 500mg/kg) ($P<0.05$-0.001) when compared with untreated control. Serum creatinine concentration was significantly decrease in goats treated with *J.curcas* (250-500 mg/kg) ($P<0.001$) when compared with untreated control, there is no significant difference in creatinine concentratin in Ivermectin group compared with untreated control. Serum bilirubin concentration showed significant increase in group A which treated with (250mg/kg) *J.curcas* ($P<0.001$) when compared with control group, goats treated with Ivermectin showed significant decrease in bilirubin concentration when compared with untreated control. There is no significant different in bilirubin concentration in group B which treated with 500mg/kg *J.curcas* when compared with control group. Serum iron concentration showed significant increase in all treated groups ($P<0.001$) when compared with untreated control.

# CHAPTER FOUR
## DISCUSSIN


The results of the present study indicated that the local breeds of goats in Southern Darfur are fairly susceptible to the experimental infection with infective *H. contortus* larvae at dose levels of 125 larvae / kg. bwt. The main clinical signs were depression, weakness, emaciation and loss of appetite. These findings were similar to those previously reported for the natural experimental course of the disease **(Fabiyi *et al.*, 1979**; **Idris, 1980; Eysker and Ogunsusi, 1980; Jubb *et al.*, 1985, Blood and Radositis, 1989; Abakar, 1996; Omar, 1999 and Ismail, 2002).** The present study showed that *Haemonchus contortus* responded to the administration of *Piper abyssinica* at doses 500 and 1000 mg/kg b .wt and showed 18.6 and 57.4% efficacy rates respectively and the reduction in worm numbers was 388 and 203 respectively. However the effect of the powdered seeds at the high doses (1000mg/kg) is higher than that produced by *Jatropha cucras* which showed efficacy rate 8.10 and 43.3 % at doses 250 and 500 mg/kg respectively and it appeared that the response of *Haemonchus contortus* to *Piper abyssinica* powdered seeds was positively correlated with the dose and no toxic effect occurred at the doses used.The genus Piper contains a number of active constituents such as pipradine alkaloid **(Ahn *et al.,* 1992)** phenyl propanoids **(koul *et al*., 1993),** phenolic compounds **(Chiranjib *et al*., 1990)** and other active constituents. The nematodal activity of *Piper abyssinica* is likely due to these constituents collectively or separately. It was reported that plant constituent of *Vernonia amygdalina* which include vernodolin and vernolepin **(Al-Magboul *et al.,* 1997)** has high anti schistosomal activity

when tested in vitro. **Ajayi *et al.*, (1990)** reported that the water extract of the same plant *V.amygdalina* at concentrations of 25% and 5% controlled the nematode on the laboratory animal. The anthelmintic activity of *Pipr abyssinica* against experimental infection of tapeworm *Raillietina tetragona* in chicks was evaluated by **Osman (2001)** and found significant result, 71.5 and 87.5% efficacies at doses 250mg/kg and 500mg/kg b.wt.. The ethanolic extract of *Azadirachta indica* gave significant result against gastro intestinal nematodes *H.contortus* in the labrotray **(Hordegen *et al.*, 2003).** (**Kahiya *et al.*, 2003)** reported that *Acacia nilotica* and *Acacia karro* have anthelmintic effects against *H.contortus* in goats.

The experiment with *J.curcas* showed that the administration of powdered seed of *Jatropha curcas* at doses 250 and 500mg/kg against *H. contortus* infection in goats for two consecutive days produced 8.1 and 43.30% efficacy rates respectively. However the effect is less than that produce by Ivermectin at 200 µg/kg. b.wt showed efficacy 99.5%. **Oliver - Bever (1986)** reported that the seed of *Jatropha curcas* contains toxalbumin, curcin, glacturonic acid and glycoside, and accordingly **Adam (1978)** showed that *Jatropha curcas* seed is active against strongloides infection but not against *Haemonchus contortus* infection in goats. **Idris *et al.*, (1982)** found that when investigated *Arteimisia herba alba* shoots against *Haemonchus contortus* in goats with 2, 10 and 30 g in two goats out of six goats treated, egg production was not completely suppressed and a few adult *Haemonchus* worms were found in the abomasums. **Asprey and Thornton (1955)** found that the fruit of *A. squamosa* has been described to have anthelmintic properties. **Jovellanos (1997)** reported a reduction of 95% in feacal egg count in cattle treated with a bolus of dried *A. squaosa* leaves.

The anthelmintic effect of powdered shoots of *Artemisia herba – alba* (Asteraceae), was investgated against *Haemonchous contortus* in goats and give successful results **(Idris *et al.*, 1982)**. *Nauclea latifolia* that has anthelmintic effect against ovine nematodes in Nigeria, was mentioned by **Onyeyili *et al.*, (2001).** The essential oil of *Ocium sanctum* and eugenol has anthelmintic activity **(Asha *et al.*, 2001).**

A decrease in body weight was observed in *Haemonchus contortus* infected goats in the present study. This was in agreement with many previous findings **(Idris, 1980; Jubb *et al* ,1985; Al Quaisy *et al*, 1987 ; Abakar, 1996 and Omar, 1999)**. This decrease of body weight in *Haemoncus contortus* infected animals may be due to anoroxia which results in decrease of food in take.

The prepatent period in *Haemoncus contortus* infected goats in the present study ranged from 17 to 18 days in all of the infected groups. This is in agreement with the previous results of **Hunter and Mackenzie (1982), Abakar, (1996), Omar (1999) and Ismail (2002).** In haematological parameters in goats treated with *P.abyssinica* and *J.curcas* in all groups there was significant increases in Hb, PCV and RBCs in all plant treated groups and more increase in Ivermectin treated group, and this may indicate the improvement in condition of the animal treated and the reduction in the number of worms.

The biochemical finding in goats treated with *P.abyssinica* and *J.curcas* showed significant increase in the concentration of total protein, albumin and iron and this my indicate an improvement due to the treatment and reduction of worm burden while the concentration of urea and creatinine was fluctated and bilirulin concentration is decreased in Ivermectin treated groups and *P.abyssinica* treated groups. Decrease in creatinine concentration

may be possibly due to some principles in the plant that cause for break down  of creatinine, facilitated its decrease or prevented its  formation. This was also observed by **El Asha (1997); Al – Khazraji *et al.,* (1993)** reported that there were no side effects recorded during or after treatment with *Arteimisia herba – alba,*  and that the extract of aerial parts of the same plants seem  to have minimal  adverse effect.

# CONCLUSION AND RECOMMENDATIONS

1- This study had  investigated the activity of two medicinal plants *Piper abyssinica* and *Jatropha curcas* against experimental *H.contortus* infection in Southern Darfur Desert  goats. Successful experimental infection reveal susceptibility.

2- A high  reduction in worm number was possible with a larger dose of the powdered seed of *Piper abyssinica,* which showed efficacy rates of  18.6, and 57.4% at doses 500 and 1000mg/kg b.wt.  respectively. More investigations are needed in this respect.

3- Ivermectin was highly effective against the strain of *H.contortus* used experimentally to infect animals, indicating that this strain was highly sensitive to the drug.

4- The powdered seeds of *Piper abyssinica* has higher effect against *H.contortus* than powdered seeds of *Jatropha curcas*.

5- *Jatropha curcas* had low anthelmintic activity against *Haemonchus contortus* at the doses used here and more investigations are needed furtherly.

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