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A COMPARATIVE STUDY ON THE EFFICACY OF SOME ETHIOPIAN TRADITIONAL TAENICIDES AS TESTED ON THE EARTHWORM

by

Geraldine Masese
Department of Biology
Science Faculty

Approved by:

Dr. Fisseha H. Meskal
Advisor

Dr. Seyoum Taticheff
Examiner

Dr. Beyene Petros
Examiner

Dr. Teferi Gemetchu
Examiner

A COMPARATIVE STUDY ON THE EFFICACY OF SOME ETHIOPIAN TRADITIONAL TAENICIDES AS TESTED ON THE EARTHWORM

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ABSTRACT

Intestinal parasitic infections represent serious medical and public health problems in many developing countries especially in the tropical regions. Taeniasis and cysticercosis have been recognized as important problems not only because of their impact on health but also because of economic losses resulting from condemnation of infected carcasses. The most frequently sold traditional medicines in Ethiopia are the taenicides. This is apparently due to the high prevalence of taeniasis in this country. Although a number of writers have mentioned a list of traditional taenicidal drugs in Ethiopia, very little serious work has been carried out to determine their relative efficacy. The present study is a contribution towards that end.

Twelve traditional drugs used against human taeniasis in Ethiopia were screened for their taenicidal properties using the earthworm. The drugs were extracted using water and ethanol. The potency of the traditional drug extracts was compared with that of a commercial taeniacide, niclosamide. Results showed that Hagenia abyssinica (Kosso) is the most potent traditional taeniacide, followed by Glinus lotoides (Mettere), Albizia anthelmintica (Musenna), and, Myrsine africana (Ketchemo) in that order.

1. INTRODUCTION

Taeniasis is a non-fatal intestinal infection in man caused by the adult tapeworm of the genus Taenia. According to The World Health Organization,(WHO, 1987a), the two main tapeworms of man are Taenia saginata and Taenia solium. The larval stages of these worms occur in cattle and pigs respectively. The infestation of cattle and pigs with cysts is known as cysticercosis. Human cysticercosis due to T.solium is a serious and chronic disease which may result in death (Cook,1988). According WHO, (1983), taeniasis and cysticercosis have been recognized not only as a public health problem but also as an economic problem because of the losses incurred due to condemnation of infested bovine and porcine carcasses.

1.1 Biology of Taenia

Tapeworms of the genus Taenia (Family Taeniidae) are exceptionally large worms. The adult worm is usually 4-8 meters and rarely up to 15 meters long. The body is divided into three regions; the scolex, neck and strobila. The scolex is the anterior region and it bears suckers (Annex 1 and 2). A rostellum, in the case of T.solium, is usually armed with a double row of small and large hooks. The features of this organ are considered important in classification (Annex 2).

The neck is situated immediately posterior to the scolex and this is the site of new proglottid formation. The strobila constitutes the main part of the body and it is made up of a chain of proglottides. Anterior proglottides are immature while those at the posterior end are usually gravid (Beaver et al.,1984; Schmidth and Roberts,1981). The structure of a proglottid is shown in Annex 3.

The egg is characteristically spherical with a thin outer hyaline membrane. Inside the inner capsule lies the embryophore which is made up of keratin blocks and gives the egg its characteristic radiated appearance (Annex 4). Within these, the oncospheral membrane surrounds the hexacanth embryo or oncosphere (Soulsby,1968; Schmidt and Roberts,1981).

The cysticercus is found in the intermediate host and this is the stage that is infective to the definitive host. The cysticercus has an invaginated scolex which bears suckers (Annex 5). The intermediate host for T.solium is the pig but there have been reports of cysticerci in the brain of dogs (Mckinnon,1957; Heinz and Aron,1966), monkeys and other mammals (WHO, 1976). T.solium eggs are also infective to man.

The normal intermediate host for T.saginata is the cow, but according to WHO (1983), the reindeer (Rangifer tarardus) too has been reported to serve as an intermediate host for T.saginata in northern Siberia. Reports are also available on the presence of unarmed cysticerci in llamas, pronghorn, Oryx, antelopes, bushbucks, gazelles, wildebeest and giraffes (Nelson et al., 1965; Fay, 1972; Stevenson et al., 1982 and Beaver et al., 1984). Man is not normally regarded as a host for the larval stage of T.saginata but unarmed cysticerci have been found in the human body (Fay, 1972; Pawlowski and Schultzi, 1972), although the occurrence of this and cysticercosis due to T.saginata is still an open question (Pawlowski and Schultzi, 1972).

Infections due to T.saginata and T.solium are maintained in nature with man as the only definitive host. Their life cycles are entirely dependent on the link between man and cattle in the case of T.saginata, and man and pigs in T.solium such that any break in the links can result in the total elimination of the organism (WHO,1983). A sketch of the life cycle of T.saginata and T.solium is given in Annex 6.

The adult tapeworm occurs in the jejunum of man who acquires the infection by the ingestion of raw or undercooked parasitized beef in the case of T.saginata or pork in the case of T.solium. The embryo hatches and becomes activated under the influence of gastric and intestinal juices and penetrates the intestinal mucosa to reach the general circulation. The embryos develop in various muscles. According to Ginsberg (1956), the order of frequency of infection in cattle is : the shoulder muscles, heart muscles, masseters, adductor muscles, oesophagus and psoas muscles. These sites were regarded as the most important predilection sites but several studies have, since then, shown that cysticerci are found dispersed throughout the musculature or any other organ and that there are no specific sites of predilection (see Annex 7). The distribution of T.solium cysticercus is shown in Annex 8.

1.2 Pathogenesis and symptomatology

According to Pawlowski and Shultzi (1972), infection with T.saginata rarely causes clinical problems except the irritations caused by the passage of proglottides which is noticeable in 98.3% of the patients. When other symptoms do occur, they include the following : abdominal pain (35.6%), nausea (34.4%), weakness (28.4%), weight loss (21.0%), increased appetite (17%), headache (15.5%), constipation (9.4%), dizziness (8.2%), diarrhoea (5.9%), pruritis (4.5 %) and excitation (3.4%). The gravid proglottides of T.saginata can sometimes travel to different organs (appendix, uterus, bile ducts and nasopharyngeal pathways) causing disorders related to the location of the parasite (Pedro and Boris, 1980). In areas where malnutrition is widespread, the presence of T.saginata in the intestinal tract may play a role in vitamin deficiency . In the tropics, T.saginata often occurs with other species of parasites, and under these circumstances, the effect of taeniasis can be severe (Barret-Connor and Beck, 1971). According to WHO (1967), multiple infections as well as mixed infections of T.saginata and T.solium can occur.

The pathology due to T.solium taeniasis is usually less obvious than that of T.saginata. When symptoms occur, they are similar to those of T.saginata taeniasis (Pedro and Boris, 1980). Taeniasis is diagnosed by the presence of proglottides and/or eggs in faeces.

The infection of the intermediate host by cysticercus is called cysticercosis. Cysticercosis has been recognized as an important economic problem because of the losses incurred in livestock production due to this infection. Such losses may result from the treatment of infected animals or the total condemnation of the carcasses (Pedro and Boris, 1980). According to Grindle (1978), Botswana and Kenya lost 0.5 and 1.0 million pounds respectively in the year 1976 due to bovine cysticercosis. Mexico lost an equivalent of 68.5% of the investment in the pig industry in 1986 due to porcine cysticercosis (Flisser, 1988).

Light or moderate cysticercosis in cattle and pigs is usually not associated with any defined clinical picture. Heavy infections in cattle, for example those induced experimentally, may give rise to fever, weakness, profuse salivation, anorexia, high temperature (McManus, 1963 and Soulsby, 1968) and may cause death due to a degenerative myocarditis (Soulsby, 1968). Presently, no immunodiagnostic technique is completely reliable and diagnosis is by the detection of cysts in the carcass during meet inspection (Grindle, 1978).

T.solium cysticercosis in man is a serious and chronic disease which is often fatal because of the frequent cerebral location of cysticerci. Cysticercosis is the most common parasitic cause of neurological diseases in man (Cook, 1988). According to WHO (1983), there are three basic groups of human cysticercosis whose clinical pathology depends on the number, localization and stage of development of the parasite and the individual host reaction. These include ocular cysticercosis, neurocysticercosis, and muscular cysticercosis. Immunological assays and computed tomography are used in diagnosis (Flisser, 1988).

1.3 Epidemiology

The occurrence of T. saginata is global but the infection is particularly important in Africa, Latin America and some Mediterranean countries. In 1947, it was estimated that 38.9 million people had T.saginata while 2.5 million had T.solium throughout the world. In Africa alone, 12 million people had T.saginata while 0.5 million had T.solium (Stoll, 1947). It is thought that since then,the number of infected persons has increased along with the increase in human and animal populations (Pedro and Boris, 1980). The prevalence of taeniasis in man is not well known because the condition is mainly subclinical and the available information is based on isolated studies of specific sectors of the population,for example school going children and recruits to the armed forces.

Studies carried out between 1970 and 1980 showed the following rates of infection caused by T.saginata: USA 0.02%, Cuba 0.1%, Argentina 0.6%, Chile 1.6%, Guatemala 1.7% and Brazil 1-2% (Pedro and Boris, 1980). It is speculated that the infection caused by T.saginata may possibly be on the increase in Europe because of the growing predilection for undercooked steaks. Another factor that has influenced the increase of taeniasis in recent years is the increasing use of detergents that impede the natural destruction of the eggs in sewerage systems (WHO, 1987).

According to WHO (1983), the prevalence of T.saginata in man can be roughly classified into three groups:

1) Highly endemic areas where prevalence in the country or region exceeds 10%, and this includes some African countries like Ethiopia where the prevalence is 30%, Uganda 16%, Swaziland 14% and Kenya 20% (Grindle and Odupoy, 1977). According to WHO (1983), T.saginata is also endemic in south-central republics of the former USSR and some mediterranean countries such as Syria, Lebanon and Yugoslavia.

2) Areas with moderate infection rate with the prevalence exceeding 0.1% but less than 10% are characterized by the existence of a small number of human carriers and a wide dispersal of eggs in the environment. Europe, Thailand, India, Vietnam, Philippines, Japan and South America fall in this category. According to WHO (1967), the prevalence of cysticerci in some European countries ranged between 0.25 and 1.26%.

3) Areas with a prevalence below 0.1% or even free of T.saginata taeniasis include the USA, Canada, Australia and some Western Pacific countries. Epidemic cysticercosis and taeniasis are characterized by the feedlot situations, for example, in the USA and Canada

and transmission is affected by the standards of hygiene of the farm workers. Poor toilet habits by a single carrier led to a massive outbreak of cysticercosis in a USA ranch (McAnish, 1974).

The epidemiological situation under which the parasite occurs is particularly important in the transmission of T.saginata. In a hyperendemic area, transmission is affected by eating raw meat and lack of sanitation. An example of this is Kajiado in Kenya where a random stool examination showed that 15% of the population had taeniasis and 20% of the cattle had cysticercosis (Grindle and Odupoy, 1977).

In the endemic situation, transmission is affected by poor sewage systems and inadequate sewerage treatment (WHO, 1983). The third category in endemicity is the epidemic areas where transmission is affected by the inadequate treatment of sewage and the deliberate use of sewage in irrigating pasture (WHO, 1983). In the case of T.solium, with modern pig husbandry methods, it is unlikely that epidemics of taeniasis and cysticercosis can occur but where pig rearing procedures are still primitive, poor standards of hygiene favor epidemic outbreaks (WHO, 1983; WHO, 1987).

According to WHO (1987), control measures include the improvement of general sanitation, health education, pig husbandry and meat inspection.

1.4 Treatment of taeniasis

The main drugs for treating tapeworms in the 1950's were derivatives of acridine, mepacrine hydrochloride, extracts of an unnamed male fern and tin compounds. In 1956, dichlorophane was introduced followed by niclosamide in 1960. Dichlorophane, trichlorophane and bithianol were previously used but are no longer recommended due to their side effects (WHO, 1983). Presently, the drugs used in tapeworm infections in man are niclosamide and praziquantel.

Niclosamide is claimed to be the drug of choice with a high level of safety. Many reports confirm that there are very few side effects related to the use of this drug. When side effects do occur, they include nausea, vomiting and abdominal pain (Abrams et al., 1963; Farahmandian et al., 1973; Pawlowski and Shultzi, 1972). Niclosamide is believed to act by inhibiting oxidative phosphorylation in cestode mitochondria. The scolex gets detached from the intestinal wall by contact with the drug and this results in killing the parasite. The dead worm is digested within the intestine and neither the scolex nor the proglottides can be identified in the stool even after purgation.

The strong efficacy of praziquantel for cestode infections was first reported in 1975. It is used extensively for the treatment of schistosomiasis and other trematode infections of man. A complete dose for an adult is 600mg, and half this amount for children. The mode of action of praziquantel is through the paralysis of the worm and/or damage of the tegument which results in the exposure of the worm to attack by proteolytic enzymes whose action on the worm results in loss of attachment and expulsion of the worm. The other effects of praziquantel include changes in carbohydrate,protein and nucleotide metabolism, decreased enzyme activities and changes in the surface membrane. All these factors lead to the destruction and subsequent elimination of the parasite (Harnet, 1988).

Other drugs that have shown cestocidal effects are antibiotics such as paromomycin whose cure rate is about 90% in the treatment of T.saginata taeniasis (Wittner and Tanowitz, 1971). Mebendazole (vermox) which is extensively used in nematode infections has also been reported to act on the two forms of taeniasis (Vakil et al., 1975).

Although it is estimated that 97% of the total population uses traditional taenicides, Ethiopia is among those countries in the world that import large amounts of commercial taenicides (Pankhurst, 1968). Information collected from the Central Medical Stores of the Ministry of Health shows that in the first ten months of 1992/93 fiscal year, the Transitional Government of Ethiopia spent about 10.5 million US dollars to import various anthelmintics and out of this, 1.3 million was spent on taenicides. The two taenicides imported into Ethiopia are niclosamide and dichlorophane and the amount of money spent on each one of them in the period stated above was 1.0 and 0.3 million US dollars respectively. The figures given are only those that cater for government institutions and does not include drugs brought in by private companies and relief agencies. In the above period, 21,000 packs of niclosamide,each containing 500 tablets were imported while the amount of dichlorophane imported was 8,400 packs each containing 1000 tablets. Since taenicides are freely sold without necessarily having a prescription, the consumption of these drugs is much higher than the records at the Ministry of Health indicate and even doubling this values could account for about 70% of the total consumption (Djeregna 1993 personal communication).

1.5 Justification and objectives

About 80% of the population in most developing countries depend on traditional medicine for health care (WHO, 1978). According to Kloos et al (1978), 95% of the population in Ethiopia relies on household remedies and herbal medicines of traditional healers for their health needs. Studies on medicinal plants and plant products in Ethiopian markets have shown that the most common and widely used medicinal plants are taenicides. This shows that in the herbal folklore of Ethiopia, anthelmintics in general and taenicides in particular occupy a central position (Kloos, 1976). According to Shibru (1986), the use of commercial taenicides in Ethiopia is widespread and this is said to have contributed towards the occurrence of liver problems in the country. Despite the widespread use of these traditional medicaments and the adverse effects of the commercial drugs, there is no systematic study on traditional drugs and there is still little effort made to replace expensive and sometimes highly toxic commercial drugs by traditional ones.

Out of the thirty-seven Ethiopian traditional taenicides known in the literature (see Annex 9), taenicidal activity has been experimentally demonstrated in seventeen of them, (marked by *), (Pankhurst, 1965; Pankhurst, 1968; Berhanu et al., 1976; Berhanu and Ermias 1978; Fisseha et al.; 1981 Mesfin, 1986). There is need to determine the relative efficacy of these drugs. H.abyssinica (Kosso) is said to be the most effective of these taenicides but there is shortage of scientific evidence,for example the Lc50 values, on the efficacy of the drugs.

Studies on the effect of various taenicides on *T.saginata* are limited. Toxicity of some of the taenicides on *T.saginata* have been carried out (Chernishov et al., 1978). From the literature available,the effect of the drugs on the cysticercus is not yet known. Except for *A. anthelmintica* (musenna), the Lc50 of the various taenicides has not yet been experimentally determined. Such information is important because it is known that an overdose of some of these drugs, for example kosso, is highly toxic (Chernishov et al., 1978). There is a need, therefore, to carry out further studies on these taenicides to determine the Lc50 and form a basis for further work towards the determination of the required dose.

Scientific research should be carried out to determine the potency,appropriate dosages and improved traditional method of preparation for efficacy and safety. Crude preparations and extracts should first be thoroughly and systematically examined before attempting refined isolations of single ingredients (WHO, 1978).

The purpose of this study is to contribute towards systematic studies on traditional taenicides accessible during the study. The broad objectives of the present study is to determine the efficacy of the most commonly used traditional taenicides compared with a commercial taenicide.

The specific objectives of the project are:

a) To find out the various taenicidal drugs available in Merkato (an open market in Addis Ababa), how they are used, and their popularity.

b) To determine the toxicity of the various concentrations of the taenicides on the cysticercus, adult tapeworm and the earthworm.

c) To determine the Lc50 of the traditional taenicides and compare it with that of a commercial taenicide.

d) To compare the relative efficacy of the different herbs found to be effective taenicides in the present study.

e) To determine the most efficient and readily available method of extracting the taenicidal compounds.

2. MATERIALS AND METHODS

Market surveys and laboratory experiments were carried out in the present study.

2.1 Market surveys

A total of five visits were made to Merkato (the largest open market in Ethiopia) in Addis Ababa. Anyone selling traditional medicinal plant parts, either in the open or permanent stalls was considered as a drug vendor. Information was collected from the vendors by the use of questionnaires (see Annex 10) which were translated to Amharic by a guide. Particulars of the vendors were taken during the first visit so that drugs were bought from the same vendors in subsequent visits. This was done to avoid variation due to the source of raw material. Drugs were purchased from two areas of the market known as the "chat terra" and the "kibe terra". A total of ten drug vendors were interviewed. The choice of the drug vendor to be interviewed depended on the willingness of the vendor. The drugs collected were put into paper bags and labelled using the vernacular names and/or scientific names where possible. Some specimens were taken to the National Herbarium, Addis Ababa University, for identification. During the first visit, the amount of each drug bought was that which the vendor recommended as a full dose. In subsequent visits larger amounts of each drug were purchased for preparation of various extracts for screening.

2.2 Laboratory experiments

2.2.1 Preparation of traditional drug extracts

a) The drugs purchased were sundried before grinding. Barks and large seeds were first pounded using a local mortar and pestle (mukecha) to break them into manageable parts that would go through a grinding mill. To avoid contamination of one drug by another,the mortar and pestle were thoroughly washed with tap water after each sample had been pounded.

The samples were then ground using a grinding mill, (Straub model 4E,UK). The powder was collected and put into labelled vials. After preparing each sample, the mill was opened and cleaned with water and then dried using a clean dry piece of cloth.

b) 5 grams of the powdered plant material were suspended in 50ml of distilled water and the volume was made to 100ml. This was allowed to stand for one hour,and, for twelve hours and the supernatant decanted to form the stock solution. Serial dilutions of the latter were prepared and the minimum concentration that killed the experimental worms in three hours determined. For hot water extraction, 5 grams of the powdered plant was suspended in 50ml of distilled water, the volume made to 100ml and allowed to boil for 10 minutes. The supernatant was cooled and used as above.

c) For ethanol extraction, 5 grams of the powdered plant material was put into a conical flask and 200ml of alcohol was added. This mixture was then left on an automatic shaker (model GFL 3020,Germany) for 24 hours. The extract was filtered using Whatman paper number one, and the filtrate dried on Rotavapor (model RE 121,Germany). The extract was placed in a 100ml volumetric flask to which distilled water was added upto the 100ml mark. This was considered as the stock solution and serial dilutions prepared from it until the minimum concentration that killed in three hours was obtained.

d) Four tablets of niclosamide (a complete dose and an equivalent of 2.5 grams of the active substance) were suspended in 100ml of distilled water. Serial dilutions were prepared and screened until the minimum concentration that killed in three hours was obtained. This was used as a positive control.

Plans were to lyophilize the drug suspensions/extracts in order to prepare known concentrations of different dilutions of the drugs tested. Unfortunately this could not be achieved due to technical problems and we had to resort to a weaker alternative of determining the approximate percentage of dilutions of the stock solution for each drug. All attempt should be made in future to freeze-dry extracts before preparing the desired dilutions.

2.2.2 Screening method

The model organism used in screening anthelmintics is the earthworm. The earthworm method of screening drugs for anthelmintic properties was described by Sollmann (1918). According to Jenkins and Manchey (1936), all clinical vermicides are markedly toxic to earthworms. Earthworms, with regard to their great availability and ability to withstand environmental changes are well adapted to routine laboratory experimentation.

Attempt was made to screen the drugs using newly excysted larvae of Taenia saginata, but it was not easy at all to obtain infected meat from the slaughter house. This problem was also experienced by Berhanu and Ermias (1978) and Chernishov et al., (1977). All drugs were therefore screened using the earthworm method as described by Sollman (1918).

Earthworms used in this study were collected a day before use from the garden in Kabena. The worms were brought to the laboratory with the soil in which they were found. To ensure a constant supply, some earthworms were cultured in the laboratory in soil containing 20 to 30% moisture and decomposing plant materials. A plot was also prepared at the Science Faculty (Theology Campus), covered with plant material and was watered on a daily basis. This provided a further source of earthworms. To avoid variation due to different sources of earthworms, the worms used at any one experiment (including controls) were from the same source. In most cases, the earthworms used were collected from a garden near the Kabena river.

Three actively wriggling earthworms, about 5 to 8cm long were isolated and rinsed in aged tap water. They were then transferred to a clean petri dish and 25ml of the drug extract added and worm activity observed during the three hours of exposure. A worm was declared dead if it had ceased moving and did not respond to needle pricks. The worms were then transferred to another petri dish containing aged tap water for a recovery period of one hour. Each experiment was repeated three times and an average reading was taken. During all experiments a control was set up using three active worms in 25ml of aged tap water. The worms used in the control were of a similar size and from the same batch as the experimentals.

2.2.3 Determination of the Lc50

Serial dilutions of different drugs were prepared and screened until the lowest concentration that killed in three hours was obtained. Further dilutions were prepared and screened until the highest concentration was obtained in which there were no deaths. Six earthworms were used per experiment and the number of dead worms per dilution was noted after three and twenty four hours. The Lc50 was determined by using the Spearman-Karber method. According to this procedure, the $\log_{10}$ of the 50% end point dilution is obtained by simple addition of two values, the first derived from the lowest dilution of the drug suspension at which all worms were dead. The second value is related to the total number of dead worms at this dilution and higher dilution. This second value is read from mathematical tables (Lorenz and Bogel, 1973).

The calculation involved the following steps:

1) The $\log_{10}$ of the reciprocal of the lowest dilution at which all worms were dead was noted.

2) The total number of dead worms at the dilution defined in step one and at all higher dilutions was determined.

3) The numerical value attributed to the number of dead worms in step two was read from Annex 11.

4) The $\log_{10}$ of the reciprocal of the 50% endpoint dilution was obtained by adding up the values determined in steps one and three.

The Lc50 was calculated by finding the antilog of the reciprocal of this value.

3. RESULTS

3.1 Information Collected By the use of Questionnaires

Table 1 indicates the plants sold, the plant parts used and the number of drug vendors selling the drugs.

Table 1: Taenicidal drugs sold in Merkato.

Botanical name	vernacular name	plant part used	No. of vendors selling drug
Hagenia abyssinica	Kosso	flowers	10
Myrsine africana	Ketchemo	seeds	9
Croton macrostachys	Bissana	bark	8
Embelia schimperi	Enkoko	seeds	4
Lepidium sativum	Feto	seeds	7
Brassica nigra	Senafich	seeds	7
Curcubita pepo	Dubba firie	seeds	3
Glinus lotoides	Mettere	seeds	9
*Myrtus communis	Addes	leaves	

*Myrtus communis (Addes) was bought from the section of the market where butter and incense are sold. Those selling addes said that it is used only for cosmetic and not for medicinal purposes. Musenna (A.anthelmintica) was not available in the market but a sample was obtained from the National Research Institute of Health. Punica granatum (Roman) and Kalanchoe sp. (Indahulla) were collected from the Science Faculty grounds.

Hagenia abyssinica (Kosso).

This is the commonest taeniacide sold in the market with all the ten drug vendors having the plant in their collection. According to the informants, the flowers are collected, sundried, ground into a powder and drunk with water or "tala" (a local brew). According to Berhanu and Ermias (1976), only female flowers are used because the male flowers are said to be highly toxic, but this information could not be verified by the drug vendors. According to Pankhurst (1965) the drug is taken before meals and the amount taken varies from 10 to 65 grams. On average, a complete dose for an adult is about 45 grams. The drug is said to be highly effective and the demand for it is high: however, there are serious side effects if an overdose of the drug is taken. These effects range from feeling weak and nauseated to death in extreme cases (Chernishov et al., 1978). One of the remedies suggested is the drinking of milk. It is also advised that young children below ten years should not be given _H. abyssinica_. In addition old people are advised not to take this drug unless taken with _M. africana_. Children or old people are advised to take _E. schimperi_ or _C. pepo_.

Croton macrostachys (bissana):

The bark of this tree is used in treating malaria and taeniasis. The bark is sundried, ground and drunk with water. Some vendors advised that the outer bark should be removed before use, but in the laboratory, this treatment was not found to have any effect on the efficacy of the drug. According to the vendors, the demand for this drug is low and it is rarely effective unless supplemented by another taeniacide such as H.abyssinica. It is also claimed that C.macrostachys is preferred for people who are physically weak.

Glinus lotoides (mettere):

The seeds are sundried, ground and taken with water or tea. Although the demand for the drug is moderate, it is said to be very effective. An overdose of the drug may cause a general feeling of weakness and under such conditions, the vendors advice the taking of milk.

Embelia schimperi (enkoko):

The seeds are sundried and husks removed. The seeds may then be ground and taken with tea or water or they can be swallowed before grinding . In many cases, the drug is taken with tea because it has an unpleasant taste. The medicine is taken before meals and an overdose causes a burning sensation in the stomach which can be relieved by taking milk or plenty of water.

Lepidium sativum (feto):

There are two types of L. sativum sold in the market and they are designated here as L. sativum (a) and L. sativum (b). The seeds are ground and the powder is eaten with food or butter. The water extract of L. sativum (a) is light brown whereas that of L. sativum (b) is purple and the former is said to be more popular than the latter. The demand of this drug is low presumably due to the pungent smell and unpleasant taste. There are no known side effects associated with the use of L. sativum.

Brassica nigra (senafich):

According to some vendors, the seeds of B. nigra are highly effective as a taenicide although the demand of the drug is low. The seeds are ground and taken with water. An overdose of the drug causes a burning sensation which can be relieved by taking milk.

Myrsine africana (ketchemo):

This drug is normally given to children because it is very mild. The seeds are ground and taken with water as a suspension or the seed may be swallowed without chewing. The demand for the drug is reportedly low, it is rarely effective and is always given with H.abyssinica particularly for adults.

Curcubita pepo (dubba firie):

These are pumpkin seeds, and like M.africana, this medicine is basically for children. There are two methods employed in the preparation of this drug. The seeds are dried, fried and eaten or they may be ground and drunk with water.

3.2 Laboratory Results

The efficacy (kill time) of various concentrations of a one hour suspension of twelve different species of plants is given in table 2, while table 3 shows the minimum concentration of each drug that kills the earthworm in three hours. On the basis of efficacy shown in these tables,four of the drugs were selected for further screening. These include: H.abyssinica, G.lotoides, A.anthelmintica and M.africana.

Table 2. The average kill time (in minutes), of various concentrations of one hour water suspension of some Ethiopian traditional taenicides,

% Conc.	Kos	Met	Ke	Mu	Fa	Fb	ad	Sen	bis	d/f	Enk	rom
100	10±1.2	35±0.71	50±1.4	80±2.4	60±5	120±1.2	80±0.71	60±1.6	170±2.9	140±1.5	160±5	[illegible]
50	14±0.91	45±0.71	70±0.71	110±2.9	90±2	175±1.2	150±1.2	130±1.4		200±0.5	250±2.5	
25	16±1	60±0.71	125±1.2	165±5	180±2.1	240±2.3	210±0.5	205±1.4				
12.5	19±1.2	70±1.4	180±1.6	230±2.6								
6.25	30±0.71	100±2	260±2.6									
3.13	47±1.7	150±1.7										
1.56	80±1.6	185±1										
0.78	110±1.2											
0.39	166±3											
0.20	205±3.5											

Key

± standard devaation

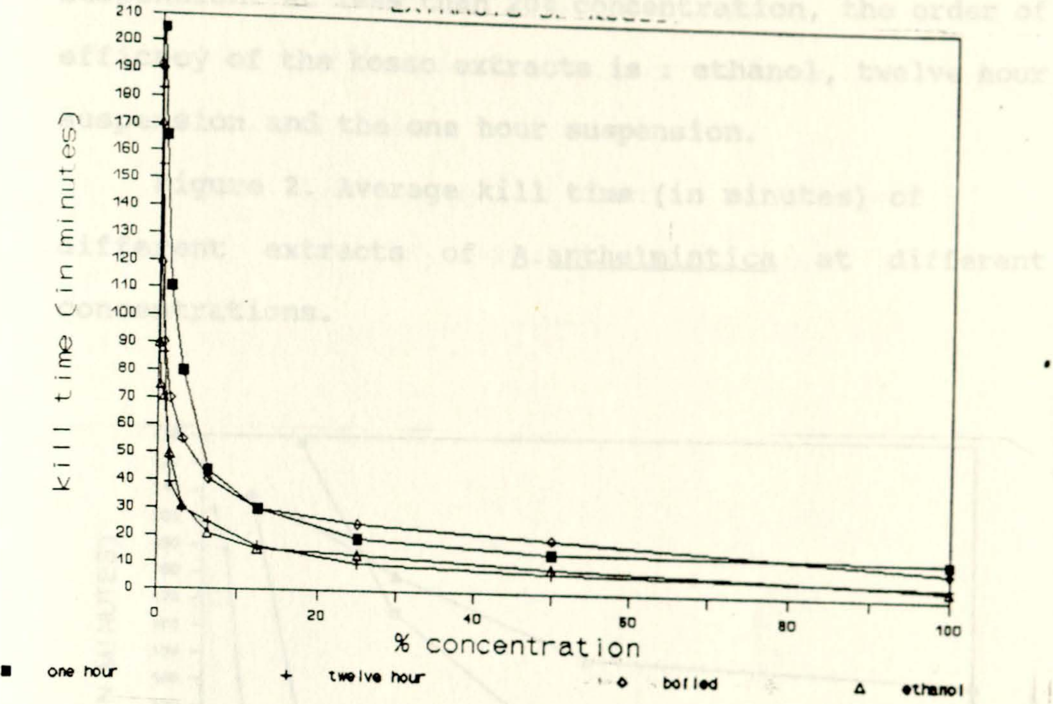
KOS	Kosso	Fb	Feto(b)	enk	Enkoko
Met	Mettere	ad	Addes	rom	Roman

Table 3: The efficacy of one hour water suspensions of different drugs as tested on the earthworm.

Drug	min.lethal conc.* in three hours.
H.abyssinica (Kosso)	0.78
G.lotoides (Mettere)	1.56
M.africana (Ketchemo)	12.5
L.sativum(a) (Feto)	25
M.communis (Addes)	12.5
A.anthelmintica(Musenna	12.5
L.sativum(b) (Feto)	25
B.nigra (Senafich)	25
E.shimperia (Enkoko)	25
C.pepo (dubba firie)	25
C.macrostachys (Bissana)	25

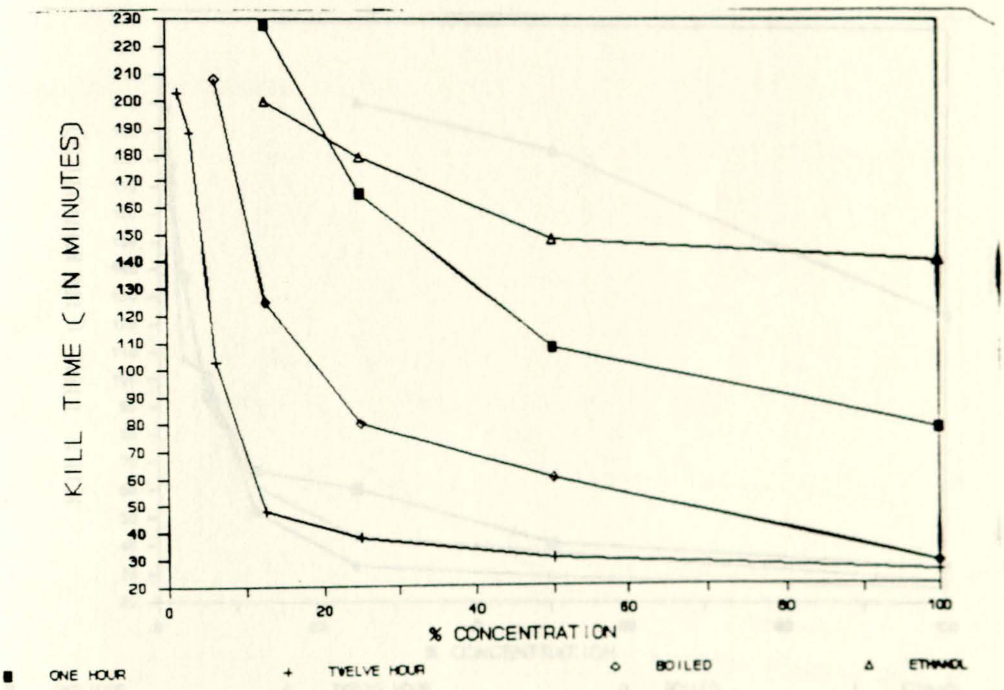
* % concentration of the stock solution.

Figure 1. Average kill time (in minutes) of different extracts of H.abyssinica at different concentrations.



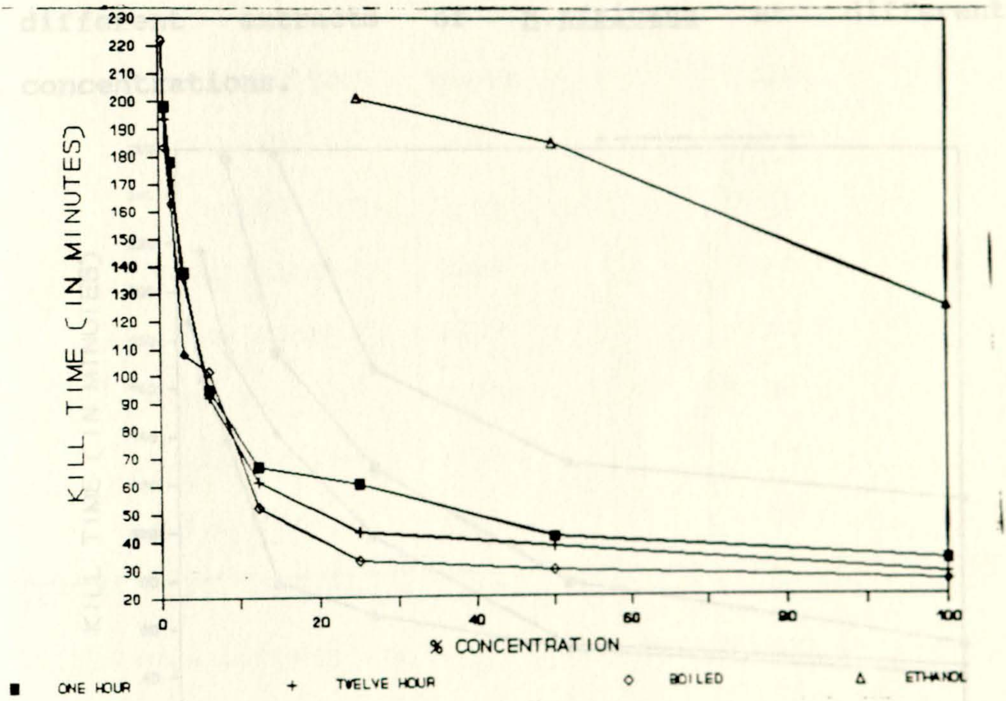
From figure 1 above, the kill time for the ethanol extract of H.abyssinica is the shortest while the boiling seems to supress the efficacy. From Annex 12, between 20% and 70% concentration, the order of efficacy of the drug preparations is: 12 hour water suspension, the ethanol extract, one hour water extract, and the boiled water suspension. At concentrations higher than 80%, the ethanol extract was the most potent followed by the twelve hour suspension, the boiled and the one hour water suspension. At less than 20% concentration, the order of efficacy of the kosso extracts is : ethanol, twelve hour suspension and the one hour suspension.

Figure 2. Average kill time (in minutes) of different extracts of A.anthelmintica at different concentrations.



From figure 2, a twelve hour suspension appears to extract the taenicidal factor of A.anthelmintica much more than the other methods, and alcohol seems to inhibit this property. At approximately the same percentage concentration the order of efficacy of the extracts is: the twelve hour suspension, boiled, one hour and finally the ethanol extract.

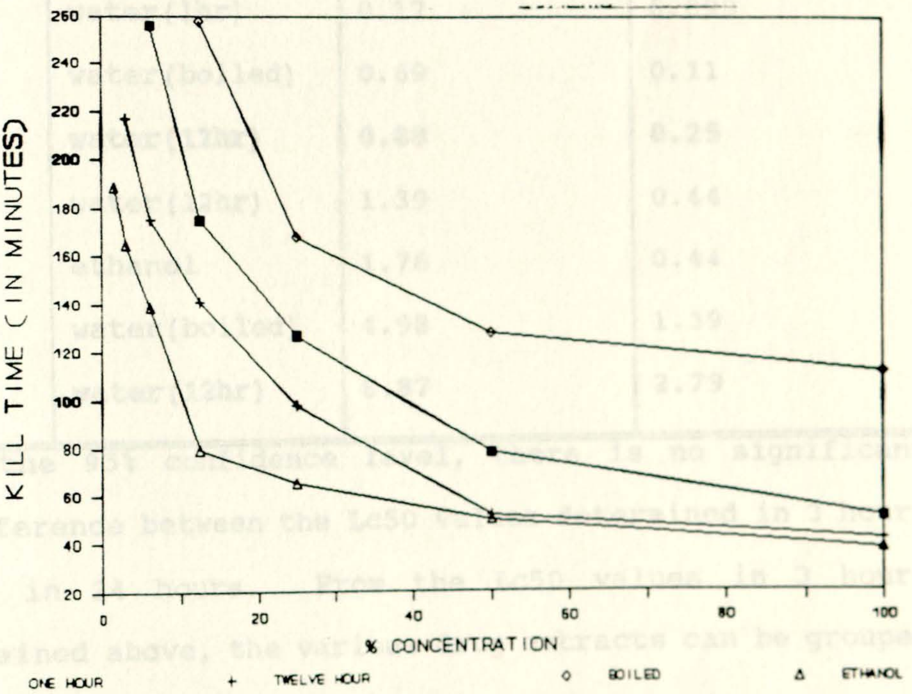
Figure 3. Average kill time (in minutes) of different extracts of G.lotoides at different concentrations.



Boiling seems to exrtact the taenicidal principle from G.lotoides much better than other methods (figure3). From figure 3, at concentrations exceeding 10%, the order of efficacy of the extracts is: the boiled suspension, the twelve hour suspension, the one hour water suspension and the ethanol extract.

From the Lc50 values,(Table 8) at the 95% confidence interval, there is no statistical difference in the efficacy of the various water extracts.

Figure 4. Average kill time (in minutes) of different extracts of M.africana at different concentrations.



From figure 4, ethanol is most effective in extracting M.africana while boiling seems to inhibit the taenicidal activity of this drug. Steeping for twelve hours increasese the activity of the drug.

able 4: The Lc50 values obtained using the earthworm method.

Drug	extract used	Lc50	values
		3 hours	24 hours
Niclosamide	water	0.004	0.001
kosso	ethanol	0.024	0.007
kosso	water(12hr)	0.087	0.031
kosso	water(boiled)	o.078	0.044
kosso	water(1hr)	0.17	0.098
Mettere	water(boiled)	0.69	0.11
Mettere	water(12hr)	0.88	0.25
Musenna	water(12hr)	1.39	0.44
Ketchemo	ethanol	1.76	0.44
Musenna	water(boiled)	4.98	1.39
Ketchemo	water(12hr)	8.87	2.79

At the 95% confidence level, there is no significant difference between the Lc50 values determined in 3 hours and in 24 hours. From the Lc50 values in 3 hours obtained above, the various drug extracts can be grouped into five classes:

Class I: % Lc50 values ranging from 0.01-0.09 and this includes the ethanol, boiled and twelve hour suspensions of H.abyssinica.

Class II: % Lc50 values ranging from 0.1-0.9. Included in this class are; the one hour suspension of H.abyssinica, and the boiled and twelve hour water suspensions of G.lotoides.

Class III: % Lc50 values ranging from 1.0-1.9 and includes the ethanol extract of M.africana and the twelve hour water suspension of A.anthelmintica

Class IV: % Lc50 values higher than 2. The drug extracts that fall in this category are, the boiled suspensions of A.anthelmintica and M.africana

DISCUSSION

A large number of the drug vendors were not willing to be interviewed and it was learnt that there is a general belief that disclosing information about the drug will reduce its efficacy or render it inactive all together. From the market surveys carried out, H.abyssinica(Kosso) is the commonest traditional herbal taenicide sold (Table 3). These findings are in agreement with those of Kloos (1976) and Kloos et al. (1978). The same survey shows that H.abyssinica is the most effective taenicide. According to Berhanu et al. (1976), the active principle of H.abyssinica is highly soluble in ethanol and it has been isolated and identified as kossotoxin. As shown in figure 1, the potency of all extracts of H.abyssinica is very close to one another. From the Lc50 values in three hours exposure, the statistical difference (at the 95% level) between the ethanol extract, the twelve hour and the boiled water suspensions is not significant thus indicating that the efficacy of these extracts is the same. There is, however, a significant difference between the efficacy of the one hour suspension and the other extracts of H.abyssinica. Of all the extracts, the one hour extract is the least potent. Traditionally, H.abyssinica is sometimes taken with "Tela" (a local alcoholic brew in Ethiopia) and it is probable that this increases the efficacy of the drug because from this study, the ethanol extract of this drug has a better performance than the water extracts.

During the study, it was observed that exposure of the earthworm to the various extracts of H.abyssinica first led to intense wriggling of the worm followed by a gradual reduction in motility and finally to complete loss of motility. The mode of action of the drug may be through strong stimulation of the musculature of the tapeworm followed by a gradual depression. The drug does not, however, kill the worm as a whole. The scolex remains viable and that is why the parasite re-establishes itself after about eight weeks following treatment (Shibru,1986). This plant is a highland species and is found at an altitude of 2,450-3,250m. In Ethiopia, the eating of semi-raw or raw beef is mainly a practice of the highlanders and this is why H.abyssinica may be the most frequently used traditional taenicide in Merkato, which is a highland location.

Kloos, (1976) includes M.communis (Addes) as one of the medicinal plant products sold in Merkato; but according to the present study, M.communis is sold only for cosmetic purposes. The disease that M.communis treats is not given by Kloos (1976), but Berhanu and Ermias (1978) show that M.communis is used against taeniasis. None of the drug vendors interviewed in the present study had any knowledge about the use of M.communis as a taenicide or any other medicinal value. In the present survey too, M.communis was not available in the "medicine market" but was found in the "butter market" where other cosmetics are also sold thus indicating its use as a cosmetic rather than medicine. From the laboratory results, the efficacy of M.communis as tested on the earthworm is low and the minimum concentration required to kill all worms in three hours is 12.5% of the stock solution as compared to 0.78% of H.abyssinica (Table 3). The performance of M.communis is, however, better than some of the common taenicides used, for example, C.macrostachys, C.pepo, and E.schimperi (Table 4).

A.anthelmintica (Musenna) was not available in the market and the drug vendors interviewed had no knowledge on the medicinal use of this drug. The use of A.anthelmintica as a taeniacide in Ethiopia has been recorded by Watt and Breyer-Bradjwick (1962) and by Fisseha et al. (1981). According to the latter workers, A.anthelmintica is used as a taeniacide at Debra Libanos where people reportedly claim that it is better than H.abyssinica as it is believed to destroy the whole worm along with the scolex. A.anthelmintica occurs in bushland at an altitude of 500-1,350m (Watt and Breyer-Bradjwick 1964) and this may be the reason why the drug is not available in Merkato, Addis Ababa since this is a high altitude area. According to Watt and Breyer-Bradjwick (1962), the active principle of A.anthelmintica is a non crystalline triterpenoid saponin which has been named Musennin. The mode of action of Musennin is thought to be through the digestion of the parasite and as a result, the latter is not ejected as a whole. As indicated by Fisseha et al (1981), this may be one of the reasons why A.anthelmintica is preferred to other taenicides for the treatment of infected children. This drug was not found to digest the the earthworm and this may due to the differences in the structure of the cuticle of the earthworm and the tapeworm.

According to Chernishov et al. (1977), G.lotoides (Mettere) is widely used because unlike H.abyssinica, it has no outward side effects. The findings from the interviews of the present study, however, show that an overdose of G.lotoides may cause nausea and/or a burning sensation. The efficacy of the ethanol extract of G.lotoides is very low (Annex 14) This is in agreement with the findings of Mulatu (1978) who established that the active principle of G.lotoides is more soluble in water than in ethanol. The active component of mettere has been identified as mettere saponinis (Berhanu, 1976). According to Mulatu (1978), only one out of the four compounds isolated from mettere saponins show taenicidal activity; further, this compound acts by dissolution of the cuticle of the worm resulting in the subsequent paralysis and expulsion of the worm. This observation was, however, not made during this study.

The ethanol extract of M.africana (Ketchemo) is more potent than the water suspensions (Annex 15). The decreasing order of efficacy of the water suspensions is : the twelve hour suspension, the one hour suspension and the boiled suspension. The anthelmintic principle of M.africana appears to be more soluble in ethanol than in water. Boiling seems to destroy the anthelmintic component of the drug thus rendering it less potent. From the Lc50 values, the potency of the ethanol extract does not significantly differ from the twelve hour suspension of A.anthelmintica.

C.macrostachys, whose bark is used to treat taeniasis and malaria was also common in the market and was being sold by 80% of the drug vendors interviewed. According to some of the drug vendors interviewed, the outer part of the bark should be removed before preparing the drug. Experiments carried out in the laboratory, however, show that this kind of treatment has no effect on the efficacy of the drug either on the earthworm or on the nematode. According to Watt and Breyer-Bradjwick (1962), The bark of C.macrostachys contains crotin but this has not been established as the active principle against helminths. The efficacy of C.macrostachys as tested on the earthworm is low and the minimum concentration that kills the worms is 100% of the stock solution as compared to 0.39% of H.abyssinica (Table 2). C.macrostachys, though not very effective as a taenicide, is popular because it is believed to serve as a prophylaxis against malaria. According to Watt and Breyer-Bradjwick (1962), the Chaga tribe of Tanzania use the fresh leaf juice together with the leaves of E.schimperi as an anthelmintic.

The information collected from the drug vendors indicates that L.sativum (a) is almost as effective as H.abyssinica but it's demand is low. The demand for L.sativum in general is low and this may be because it has a pungent smell and a sour taste. Berhanu and Ermias (1978) rank this drug as the most effective taenicide. The findings of the current study, however, indicate that the efficacy of L.sativum comes after H.abyssinica, G.lotoides, and M.africana (Table 3).

The finding of Berhanu and Ermias (1978) are also contrary to the information collected from the drug vendors who indicated that H.abyssinica is the most effective taenicide. Similar results were obtained by Kloos (1976) and Kloos et al. (1978). The different procedures adopted for extracting the drugs and the solvents used by Berhanu and Ermias (1978) might account for the contradictory results.

There are different methods employed in the preparation and use of traditional medicine. Some of the procedures employed include boiling, overnight steeping or just suspending the drug for a few minutes in water before taking. In practically all methods of extraction, H.abyssinica comes out to be most effective. The same also applies to G.lotoides except that the ethanol extract is far inferior to the others. For A.anthelmintica, long hours of suspension appear to be superior while ethanol does not seem to extract much of the active principle. Alcohol seems to act better on M.africana in extracting its taenicidal principle, while boiling in water has little effect (Annexes 12-15).

Except for Kalanchoe sp., all the twelve drugs tested showed some degree of activity within three hours. There is need to carry out further studies on these drugs in an effort to develope appropriate dosages inorder to promote their usage.

Traditional medicine in Africa has been practised for a long time and caters for the medical health requirements of about 70-80% of the population (WHO 1976). There is need to develop and promote the use of these traditional medicaments as this could go a long way in providing majority of the population with health care and save on the large amounts of money spent on importing western medicaments.

According to Abrams et al., (1963), niclosamide acts by inhibiting oxidative phosphorylation in cestode mitochondria. The scolex and proximal segments are digested by contact with the drug and this results in the separation of the scolex from the intestinal wall. The dead worm is digested within the intestine and neither the scolex nor the proglottides can be identified in the stool even after purging.

Cestode infections may induce type II hypersensitivity reactions. The production of antibodies due to the presence of the worm may lead to immune complex disorder. The antibody involved is IgE or IgA and the resultant antigen- antibody complex may exacerbate the infection. Some of the drugs used in helminthe infections may also induce these kind of reactions (Jawetz et al., 1974). Traditional medicaments may not have some of these undesired effects. The use of these medicaments require further investigetions especially in relation to host reactions.

The earthworm has been used for many years as the test organism for anthelmintics and the method employed was described by Sollmann (1918). Studies carried out by Sollman (1918) and Jenkins and Manchey (1936) showed that all clinical anthelmintics are toxic to the earthworms and according to their report, a substance that is not toxic to earthworms is not worthy further trial. The earthworm method is also favorable because the technique is simple and the worms are readily available. However, the use of the earthworm as a model for screening drugs for their anthelmintic properties has been criticized and declared irrational by Lamson and Ward (1936) whose argument is based on the fact that the biology of the earthworm is completely different from that of intestinal helminths. These authors clearly refer to Ascaris (a nematode) in comparison with earthworms and suggests that pig and dog Ascaris which are readily available should be used instead of earthworms. In the present study, a suspension of vermox (a nematocide), containing 10 tablets in 100ml of distilled water,was screened using the earthworm method and it was found that vermox did not kill the earthworms after 24 hours. From this observation, it may be possible that earthworms are not good models for screening for nematocides but can be used for screening for taenicides. However, further works should also consider the use of freshly excysted T.saginata. Cysts of this worm can be obtained from the Addis Ababa Abattoir if more conducive and appropriate administrative arrangement can be worked out between relevant authorities of the university and the abattoir.

5. CONCLUSION

1. H.abyssinica is the most effective and popular taenicide. The decreasing order of efficacy of the other drugs is G.lotoides, A.anthelmintica and M.africana.

2. Steeping for twelve hours increased the efficacy of H.abyssinica, A.anthelmintica and M.africana but decreased that of G.lotoides.

3. Except for H.abyssinica, ethanol extraction does not improve the efficacy of the drugs.

4. Further work should be carried out using lyophilized material so that the actual amount of the drug used can be determined.

5. Attempt should be made to use newly excysted tapeworms.

6. In-vivo experiments using mice infected with Hymenolopis nana should be carried out.

7. Information concerning the use of the various drugs should be obtained from both the users and the drug vendors.

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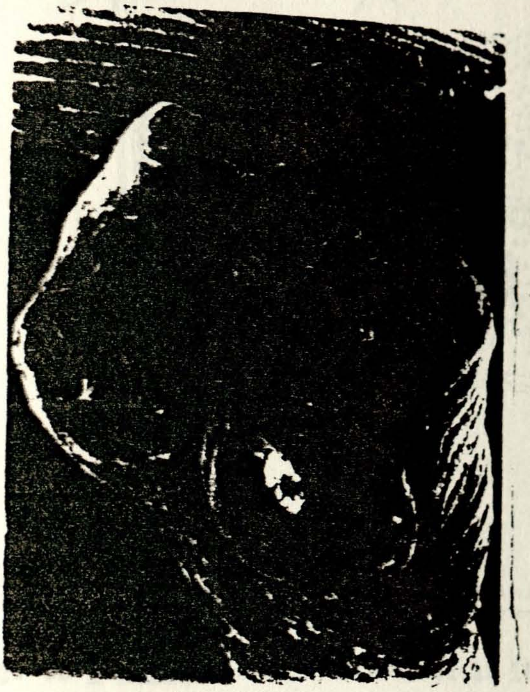
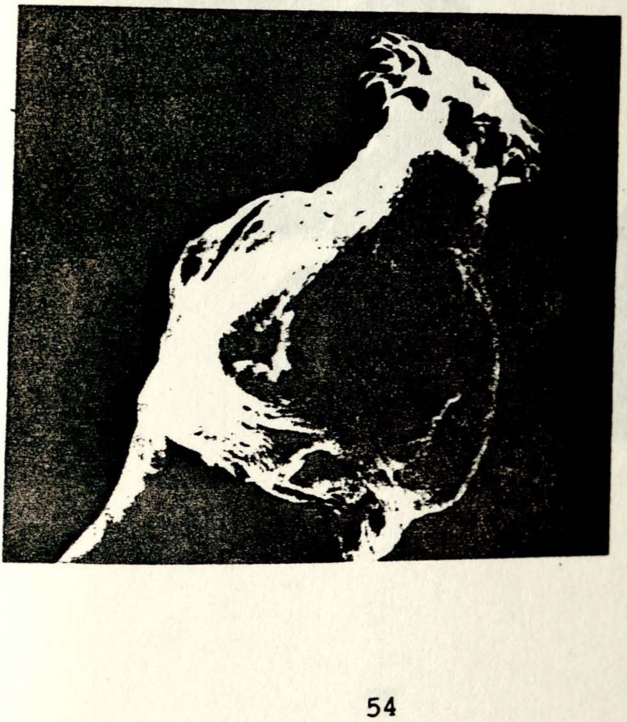
(After Flisser et al 1982).

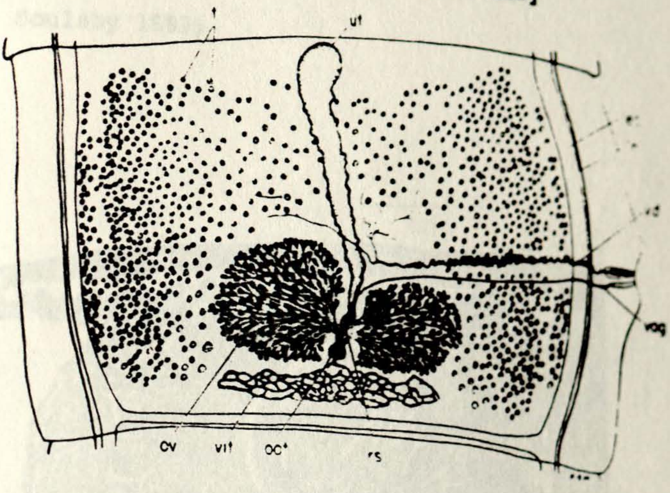


ANNEX 2. Scanning electromicrograph of T.solium scolex

(After Flisser et al 1982).



ANNEX 3. Mature progglotid of Taenia sp. (After Soulsby 1982)



lec	longitudinal excretory canal	[illegible]	testes
ln	lateral nerve	[illegible]	uterus
oot	oötype and Mehlis gland	[illegible]	vagina
ov	ovary	vit	vitellarium
rs	receptaculum seminis	[illegible]	vas deferens

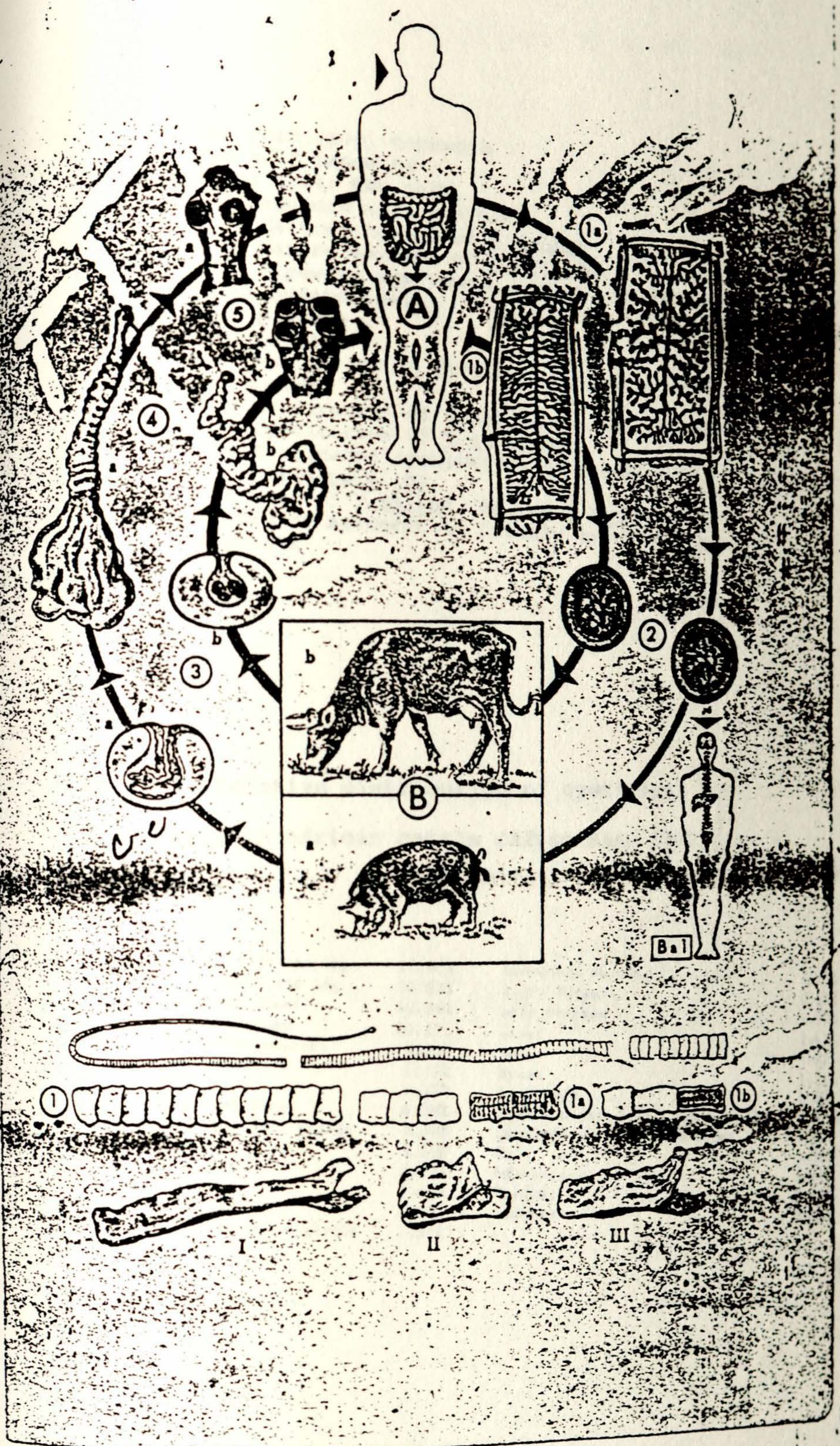
ANNEX 4. Eggs of T. solium (After Flisser et al 1982)

1

ANNEX 5. Electromicrograph of T.saginata cysticercus

(After Soulsby 1982)

ANNEX 6. Life cycle of T.solium (a) and T.saginata (b) (After Piekarski 1962)



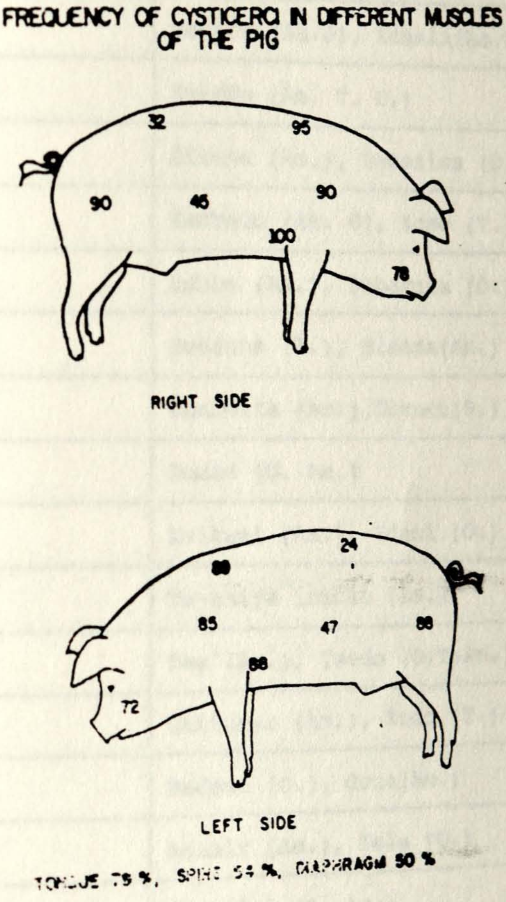
Legend for Annex 6

(a) The pork (pig) tapeworm
(b) The **beef tapeworm**
(A) Final host: **Man** only
1 Tapeworm with its head
1a **Mature** segment of T.solium
1b **Mature** segment of T.saginata
2 **Tapeworm** egg (**emryo** with six hooked larva)
(B) **Intermediate host:**
a) pig\man
b) cattle
3-4 Cysticercus in different stages of evagination of scolex
3a Cysticercus of T.solium commencing evagination
b Cysticercus of T.saginata
4 Evaginated cysticercus of T.solium (a) and T.saginata (b)
5 **head of** T.solium (a) and T.saginata(b)
I-III Phases of **movement** of freshly detached tapeworm segments

ANNEX 7. Relative distribution of cysticerci of T.saginata in African cattle (After Mann 1982).

Right thigh and leg	12.85%	Abdominal muscles	2.27%
Left thigh and leg	12.06%	Right forearm	2.12%
Back and loins	10.29%	Left forearm	1.57%
Neck and hump	10.17%	Liver	1.43%
Sublumbar muscles	8.84%	Lungs	1.43%
Left shoulder	5.72%	Hyoid	1.11%
Heart	5.35%	Diaphragm	1.06%
Right shoulder	4.39%	**Face and ext. masticatory muscles**	**0.787%**
Left shoulder girdle	**3.00%**	**Internal masticatory muscles**	0.72%
Tongue	3.00%	**Tail**	0.34%
Left arm	2.93%	Oesophagus	0.32%
Thorax	2.82%	Trachea	0.25%
Right arm	2.74%	Kidneys	0.09%
Right shoulder girdle	2.51%		

ANNEX 8. Distribution of T.solium cysticercus in heavily inf
carcass (After Vergera 1970 as cited by WHO 1983).



ANNEX 9. Traditional taenicides of Ethiopia (After Pankhurst, 1968; Berhanu and Ermias, 1978; Mesfin, 1978, Fisseha et al., 1981).

BOTANICAL NAME	LOCAL NAME
1. Hagenia abyssinica*	Koso (Am.), Ducha (O)
2. Glinus lotoides*	Metere (Am.O), Kasala(Am.T.)
3. Embelia schimperi*	Enkoko (Am. T. O.)
4. Croton macrostachys*	Bisana (Am.), Bekenisa (O.)
5. Myrsine africana*	Kechemo (AM. O), Zoso (T.)
6. Curcubita pepo	Dubba (Am.), Dabakula (O.)
7. Albizia anthelmintica*	Musenna (T.), Bisena(Am.)
8. Grewia ferruginea	Lenkwata (Am.),Dokumu(O.)
9. Punica granatum	Roman (O. Am.)
10. Opuntia ficus-indica	Kulkwal (Am.), Adami (O.)
11. Smilax goetzana	Ye-ahiya inkoko (Am.)
12. Rhamnus staddo*	Tey (Am.), Tsedo (O,T,Am.)
13. Artemisia afra*	Chikunyi (Am.), Kodo (T.)
14. Balanites aegyptiaca*	Bedeno (O.), Goza(Am.)
15. Bersama abyssinica*	Azamir (Am.), Tela (O.)
16. Brassica nigra*	Senafich (O.,Am.)
17. Calpurnia aurea*	Digita (Am.), Chekata (O.)
18. Lepidium sativum*	Feto (Am., O.), Koito (T.)
19. Maytenus senegalensis*	Kokoba (Am.), Hamakita (O.)
20. Myrtus communis*	Addes (Am.), Adisa (O.)

BOTANICAL NAME	LOCAL NAME
21.Rhus natalensis*	Embes (Am.), Dababecha (O.)
22.Sarcostemma viminale*	Yekola moyider (Am.),Hangeyu (Som.)
23.Securidaca longependinculata	Its menahe (G.)
24.Rosa abyssinica	Kega (Am.,T.), Gora (O.)
25.Oxalis trafolium	Micha (Am.)
26.Amaranthus caudatus	Lishalisho(O.), Ferengitef(Am.)
27.Amaranthus sylvestris	Gomen (Am.)
28.Chenopodium album	Amedmaho (Am.)
29.Kalanchoe sp.	Yekola-indahula (Am., O.)
30.Masea pita	Soari (O.), Kella (Am.)
31.Jasminium abyssinicum	Tamelel (Am.), Tewo (O.)
32.Celosia anthelmintica	Belbila,(Am., T.)
33.Albizia gumifera	Kachiona (T.), Imala (Am.)
34.Buddleja polystrachya	Anfar (Am.) Adodo (O.)
35.Mollugo glinus	Kossala (Am.)
36.Phytolacca dodecandra	Endond (Am.), Indode (O.)
37.Verbascum sinaiticum	Tirnaha (T.), Ketetina (Am.)

Key: O. Oromiya G. Geez Am. Amharic T. Tigrinya Som. Somali

(Adopted from Wolde, (1980)).

ANNEX 10. Questionnaire

Date.

Name:

Sex: Age: Religion:

1. What is the name of the plant? (vernacular).
2. What part of the plant is used?
3. How is the drug prepared?
4. Approximately how much of the drug is taken i.e.how many tablespoons?
5. How many times in a day is the drug taken?
6. For how many days is the drug taken?
7. What is the demand of the drug?
   a) High
   b) moderate
   c) low
8. How effective is the drug?
   a) very effective
   b) effective
   c) rarely
9. Are there any known side effects?
10. If the answer to (9) above is yes, describe these effects and the possible remedies.
11. Are there any instructions to be followed while taking the drug.
12. If the answer to (11) above is yes, what are these instructions?

Annex 11. Numerical values for calculation of titres:

Dilution(after Lorenz and Bogel 1973)

Total no. of positive animals	No. of inoculated animals per dilution n = 4	n = 5	n = 6
4	0.15		
5	0.23	0.15	
6	0.30	0.21	0.15
7	0.38	0.27	0.20
8	0.45	0.33	0.25
9	0.53	0.39	0.30
10	0.60	0.45	0.35
11	0.68	0.51	0.40
12	0.75	0.57	0.45
13	0.83	0.63	0.50
14	0.90	0.69	0.55
15	0.98	0.75	0.60
16	1.05	0.81	0.65
17	1.13	0.87	0.70
18	1.20	0.93	0.75
19	1.28	0.99	0.80
20	1.35	1.05	0.85
21	1.43	1.11	0.90
22	1.51	1.17	0.95
23	1.58	1.23	1.00
24	1.66	1.29	1.05
25	1.73	1.35	1.10
26	1.81	1.41	1.15
27	1.88	1.48	1.20
28	1.96	1.54	1.25
29	2.03	1.60	1.30
30	2.11	1.66	1.35
31	for each further positive animal add 0.075	1.72	1.40
32		1.78	1.45
33		1.84	1.51
34		1.90	[illegible]
35		1.96	[illegible]
36		2.02	1.66
37		2.08	1.71
38		2.14	1.76
39		2.20	1.81
40		2.26	1.86
		for each further positive animal add 0.060	for each further positive animal add 0.050

Annex 12: Average kill time (in minutes) of different extracts of H.abyssinica at different concentrations.

% conc.	ethanol	boiled	twelve hr	one hr
100	4±1.73	10±0.71	5±0.71	10±1.2
50	9±0.71	21±1	7±0.71	14±0.71
25	13±1.2	25±0.71	10±0.71	16±1
12.5	15±0.71	30±2.1	16±1.7	19±1.2
6.25	21±2.1	40±0.71	19±0.71	30±0.71
3.13	31±1	55±1.6	30±2.1	47±1.7
1.56	50±0.71	70±0.71	47±2.3	80±1.6
0.78	71±1.2	90±1	80±2.3	110±1.2
0.39	75±2.1	119±1	110±0.71	166±3
0.2	90±1	190±1.6	166±2.6	205±3.5
0.1	171±1.2		205±2	
0.05	192±2.3			

±, Standard deviation

Annex 13: **Average kill time (in minutes) of different extracts of** A.*anthelmintica*.

% conc.	one hr	twelve hr	boiled	ethanol
100	$80^{+}2.4$	$25^{+}2.3$	$28^{+}2.7$	$140^{+}0.71$
50	$110^{+}2.9$	$30^{+}0.71$	$60^{+}3.2$	$148^{+}2.5$
25	$165^{+}5$	$38^{+}3.5$	$80^{+}0.71$	$179^{+}1.2$
12.5	$230^{+}2.6$	$48^{+}2.7$	$125^{+}5$	$200^{+}2.7$
6.25		$103^{+}3.3$	$208^{+}2.1$	
3.13		$188^{+}2.9$		
1.56		$203^{+}3.8$		

$\pm$, Standard deviation

Annex 14: Average kill time (in minutes) of different extracts of *G. lotoides*.

% conc.	one	twelve hr	boiled	ethanol
100	35±0.71	28±2.9	25±0.71	121±1.2
50	45±0.71	37±2.6	29±1.2	186±3.1
25	60±0.71	43±2.9	33±1.7	210±0.71
12.5	70±1.4	61±2.3	50±1	
6.25	100±2	92±1.7	102±2.3	
3.13	150±1.7	137±2.9	108±2.9	
1.56	185±1	172±2.3	160±2.9	
0.78		194±1.7	180±2.9	
0.39			222±1.7	

± Standard deviation

Annex 15. Average kill time (in minutes) of different extracts of *M. africana* at different concentrations.

% conc.	one hr	twelve hr	boiled	ethanol
100	50±1.4	42±2.9	112±2.1	38±2.9
50	70±0.71	50±2.9	128±2	50±0.71
25	125±1.2	98±2.3	168±2.9	65±1
12.5	180±1.6	141±1.2	260±5	79±3.4
6.25		175±5		139±1.2
3.13		217±4.7		165±4.5
				189±1.2

± Standard deviation