European Journal of Medicinal Plants
6(2): 92-102, 2015, Article no.EJMP.2015.046
ISSN: 2231-0894

SCIENCEDOMAIN *international*
www.sciencedomain.org

Antischistosomal Activity of *Azadirachta indica* and *Ekebergia capensis* in Mice Infected with *Schistosoma mansoni*

Rael Musili[1*], Francis Muregi[2], Joseph Mwatha[1], David Muriu[1], Linus M' Rewa[1], Timothy Kamau[1], Antony Menaine[1], Simon Chege[1], Joseph Thiong'o[1], Zipporrah Ng'ang'a[3] and Gachuhi Kimani[1]

[1]Centre for Biotechnology Research and Development, Kenya Medical Research Institute (KEMRI), P.O.Box 54840, 00200, City Square, Nairobi, Kenya.
[2]Department of Biological Sciences, Mount Kenya University, P.O.Box 342-01000, Thika, Kenya.
[3]Institute of Tropical Medicine and Infectious Diseases, Jomo Kenyatta University of Agriculture and Technology, P.O.Box 62,000-00200 Nairobi, Kenya.

Authors' contributions

This work was carried out in collaboration between all authors. Authors RM and GK designed the study. Authors RM, JM, DM, LM, TK, AM, SC and JT participated in carrying out the study. Author RM performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RM, FM, ZN and GK managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/EJMP/2015/15196
Editor(s):
(1) Marcello Iriti, Plant Biology and Pathology Department of Agricultural and Environmental Sciences Milan State University, Italy.
Reviewers:
(1) Maha M Eissa, Medical Parasitology Department, Faculty of Medicine, Alexandria University, Egypt.
(2) Anonymous, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=903&id=13&aid=7640

Original Research Article

Received 12th November 2014
Accepted 16th December 2014
Published 6th January 2015

ABSTRACT

Aims: Schistosomiasis is a parasitic disease of great socio-economic and public health importance in tropical and sub-tropical countries. Praziquantel (PZQ) is the drug of choice for treatment of schistosomiasis since it is effective against all species of schistosomes. However, PZQ is less efficacious against larval stages of the parasite and there are recent concerns that long term mass drug treatment could lead to development of drug resistant strains thus prompting the need for alternative antischistosomal drugs. Plants have over the years provided a rich source of novel

**Corresponding author: E-mail: rachaelmueni@gmail.com;*

drugs for a wide range of diseases afflicting man and domestic animals.
Study Design: Swiss albino mice were infected and randomized into groups of five for plant extract treated groups, positive control groups treated with conventional drugs PZQ and artemether, as well as infected but untreated (negative control) groups.
Place and Duration of Study: The study was done at the Animal Facility in the Centre for Biotechnology Research and Development, Kenya Medical Research Institute from July 2013 to July 2014.
Methodology: Swiss albino mice were infected with 90 cercariae each and treated orally with varying doses of aqueous extracts of *Ekebergia capensis* and *Azadirachta indica* at doses of 25, 50, 100, 200 and 400 mg/kg at 2 weeks (juvenile worms), 4 weeks (immature worms) and 7 weeks (adult worms) post infection. PZQ and artemether were used as positive controls while infected untreated group was used as negative controls. Total reduction of worm load as well as egg load in the liver and intestine was used as an indicator of drug activity, relative to the infected but untreated control groups.
Results: Both *E. capensis* and *A. indica* showed significant dose-dependent percentage worm load reduction ($P<0.05$) at different doses ranging from 100 mg/kg to 400 mg/kg. These extracts also significantly reduced tissues (liver and intestine) egg load counts at doses ranging from 50 mg/kg to 400 mg/kg which was also dose-dependent.
Conclusion: The antischistosomal activity of the two plant extracts was dose dependent with *E. capensis* being more potent in reducing both the worm burden at all the stages and tissue egg load. These findings validate the potential use of medicinal plants in the management of schistosomiasis and provide a basis for exploring medicinal plants as sources for new antischistosomal agents.

Keywords: Schistosomiasis; antischistosomal agents; Schistosoma mansoni; Azadirachta indica; Ekebergia capensis.

1. INTRODUCTION

Schistosomiasis (bilharziasis or snail fever) is second only to malaria in terms of diseases that are of socio-economic and public health importance in tropical and subtropical areas [1]. The disease is endemic in 74 countries infecting more than 207 million people worldwide with 20000 deaths associated with the severe consequences of infection [2]. More than 85% of those infected live in Africa [2]. In Kenya, the infection is wide spread around Mwea irrigation scheme in Kirinyaga County, Machakos, Kitui, Taita Taveta and Nyanza [3].

The drugs that have been used in the recent past to treat schistosomiasis include metrifornate which is effective against *S. haematobium*, Oxamniquine which is effective against *S. mansoni* and artemisinin which exhibits highest activity against 1-3 week old liver stages of the parasite. Praziquantel (PZQ) has been the drug of choice for treatment of all species of schistosomes because of its efficacy, ease of administration, safety, and cost [4]. However, PZQ is only effective against adult worms and ova of schistosomes but is ineffective on immature worms that are present in recently acquired infections and this leads to reduced cure rates [5-8]. PZQ has been in use for more than two decades and possible existence of tolerant *S. mansoni* isolates have been reported in Senegal [9]. In regions of Egypt and Kenya where there has been heavy exposure to PZQ, there are reports of *S. mansoni* and *S. haematobium* resistance to treatment [10]. The effectiveness of PZQ is dependent on an intact immune system [11] and its mechanisms of action are poorly understood [7]. There is, therefore, need for development of alternatives to PZQ and herbal plants are a potential source of new drugs.

Herbal plants have been used traditionally to treat or manage schistosomiasis in Eastern and Southern Africa [12-14]. However, limited information is available on the beneficial effects of herbal preparations in the treatment and management of the disease and few attempts have been made to verify scientifically, the antischistosomal properties of such preparations. Reports of availability of plant derived concoctions for treatment of schistosomiasis from traditional practitioners in Kenya are numerous but these claims have not been sufficiently validated. Kokwaro (1993) reported that at least 19 plant species occurring in East Africa region and belonging to 10 families and 16 genera have been used traditionally as remedies for schistosomiasis. Examination of potential

of plants in management of schistosomiasis by various investigators has revealed several plants with appreciable antischistosomal activities [13-15].

Artemether, a semi-synthetic derivative of artemisinin, which is a bioactive compound derived from the Chinese herb *Artemisia annua*, has been investigated for its activity on *S. mansoni*. The drug demonstrated efficacy on early stages of the infection with 75.3-82.0% worm reduction which was boosted to 97.2-100% when the animals were subjected to various schedules of repeated treatment [16]. More recently, artemisinin derivatives, either alone or in combination with PZQ, have been shown to be effective against immature stages of *S. mansoni, S. haematobium*, and *S. japonicum* in laboratory studies and limited field studies. However, the use of oral artemisinin based monotherapy is discouraged in schistosomiasis and malaria co-endemic areas as this could lead to development of resistance to artemisinin-based drugs by Plasmodium parasites.

In the present study, we investigate the antischistosomal activity of aqueous extracts of *A. indica* (family: Meliaceae, genus: Azadirachta) and *E. capensis* (family: Meliaceae, genus: Ekebergia) against juvenile, immature adult and adult worms of *S. mansoni* in infected mice. These plants have been reported to be used traditionally for treatment of parasitic infections and abdominal complications [12].

2. MATERIALS AND METHODS

2.1 Life Cycle Maintenance of *S. mansoni*

The livers of mice were collected from *S. mansoni* infected mice; eggs were extracted from them and were hatched into miracidia. Freshly hatched miracidia (less than 1 hour old) were used to infect 30 *Biomphalaria pfeifferi* snails whose diameter was 4 mm using the routine optimized technique used for schistosome cycle maintenance at the facility. Briefly, the livers were crushed through sieves to collect eggs that were exposed to water and light in a large glass petridish to hatch into miracidia. The miracidia were picked using a Pasteur pipette into wells of a 24-well plate. Each well of the plate had 5 miracidia. The 4mm diameter snails were placed into each well and the plate was covered and left for 2 hours to give time for the miracidia to infect the snails. The infected snails were transferred into a freshly prepared and well-labelled, aerated aquarium and fed on lettuce and bone meal for 28 days at a temperature of 28-30ºC.

2.2 Infection of Experimental Mice with *S. mansoni* Cercariae

Mice used in this study were Swiss albino, males, aged 6 weeks old and weighing 20-25 grams. The infected snails were placed under a lamp for illumination to enhance cercariae shedding. Freshly shed cercariae (not more than 1 hour old) were used. Each mouse was anaesthetized using sodium pentobarbitone and infected with 90 cercariae using the abdominal ring method [17]. The infected mice were randomized into cages in groups of 5 and maintained with pellets and water *ad libitum*.

2.3 Plant Materials and Extraction

The test plants *E. capensis* and *A. indica* were collected from central Kenya (Mount Kenya Forest) and Southern Rift Valley (Nguruman Escarpment in Magadi) respectively. The plants were identified by a taxonomic botanist from East African Herbarium in Nairobi, where they were catalogued and voucher specimens deposited (*E. capensis*: Stem bark (Ec-SB/04) 26 and *A. indica*: Leaf (Ai-L/04) 10). The plant samples were then air dried at room temperature under shade and ground to powder using an electric mill. The powder was packed into one kilogram packs and stored in a dry and well ventilated room until use. The plant parts used were bark for *E. capensis* and leaves for *A. indica*. The dried chaff of the plant parts to be used (50 g of each) was weighed and soaked in 500 ml of water. This was followed by thorough mixing on a shaker and soaking for 12 hours. The mixture was then filtered and freeze dried to give at least 2g of dry solid material.

2.4 Drug Treatment of *S. mansoni* Infected Mice

Infected mice were randomized into groups of 5 and placed in separate well labelled cages. To target different developmental stages, treatments with the plant extracts were done at 2, 4 and 7 weeks post infection (p.i). to represent juvenile, immature adults and adult worms respectively. The plant extracts were administered at doses of 25, 50, 100, 200 and 400 mg/kg. Both plants extracts were administered orally by gastric gavage once a day for 3 consecutive days using an oral volume of 0.2 ml per mouse [18]. A positive control group (for 2 and 4 weeks p.i) was

treated with artemether at 200 mg/kg, administered orally by gastric gavage once a day for 3 consecutive days using an oral volume of 0.2 ml per mouse [18]. Since Praziquantel (PZQ) is known to target adult worms, another positive control group (for treatment at 7 weeks p.i) was included, where mice were orally treated with PZQ at a dose of 200 mg/kg body weight per day using a dose volume of 0.05 ml for 5 consecutive days to a total dosage of 1000 mg/kg [5]. Infected untreated negative control groups were also included that received 0.2 ml of distilled water for 3 consecutive days.

2.5 Assessment of the Effects of Treatment

2.5.1 Worm load counts

All the groups of mice were sacrificed 3 weeks post treatment by injecting them with 0.25 ml of heparinized sodium pentobarbitone intraperitoneally to euthanize them and the worms recovered through perfusion of liver and mesenteric veins [17]. Livers and guts were recovered from the perfused mice, wrapped in foil paper and stored in a freezer at -20ºC until used. The worms were collected in petri-dishes and enumerated under a dissecting microscope.

2.5.2 Tissue egg load count

The stored liver and guts were processed for tissue egg counts by trypsin digestion. Briefly, the livers and intestines were removed from the freezer and their individual weights recorded. Each tissue was chopped using a scalpel blade and the pieces placed in 200 ml beakers. Trypsin enzyme (Merck) was used to digest the tissues at 0.01 mg per gram of tissue. Phosphate buffered saline (PBS) (200 ml) was added into the beaker and the mixture homogenized using a kitchen blender into a fine emulsion. The emulsion was poured into beakers, covered with Para film and incubated at 37ºC for 2 hours with occasional shaking. After incubation, the emulsion was transferred into 50 ml centrifuge tubes and spun for 10 minutes at 2000 rpm. The supernatant was poured off leaving an egg pellet. PBS was added to help dissolve the egg pellet and the volume of the egg suspension was noted. Aliquots of 50 µl were transferred onto microscope slides using a micropipette and glass cover slips placed on top of sample. Eggs were counted under a microscope using 10X magnification. A mean was calculated and this was multiplied by 20 to estimate the number of eggs per ml. This was multiplied by the volume of egg suspension to derive the number of eggs in the whole tissue.

2.6 Statistical Analysis

The number of worms recovered from the experimental groups was expressed as mean±SD. The efficacy of the drug regimens was calculated as the mean number of recovered worms from each group relative to the control group [16]. The mean number of worms and eggs recovered from the different groups were subjected to Student's t-test using Microsoft Excel® to determine their statistical significance in comparison with the control groups. Statistical significance was set as $P<0.05$.

3. RESULTS

3.1 Treatment at 2 Weeks Post Infection

When *S. mansoni* infected mice were treated at 2 weeks p.i using *E. capensis* at a dose range of 100mg/kg to 400 mg/kg, the percentage worm load reduction as well as egg load reduction was above 50% (Table 1 and Fig. 1). The extract at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg showed worm load reduction of 57%, 67% and 76%, respectively. Similarly, egg load reduction in livers of these mice was recorded as 55%, 78% and 83% at doses of 100 mg/kg, 200 mg/kg and 400mg/kg respectively (Fig. 1). On the other hand, intestinal egg load reduction from mice for the same doses was 53%, 76% and 89% respectively. When mice were treated using *A. indica*, only a dose of 400 mg/kg gave worm and egg load reduction of >50%, which was recorded as 51% worm load reduction, with 51% and 58% egg load reduction in the liver and intestine, respectively (Fig. 1). The positive control drug, artemether at a dose of 200 mg/kg gave 88% worm load reduction, with 85% and 89% egg load reduction in the livers and intestines respectively (Fig. 1).

T-test: two sample assuming unequal variances was performed (excel data analysis tool for windows 7) to compare the statistical differences in mean worm and egg load recovered from livers and intestines between treatment groups and control groups. In the mice treated with *E. capensis* extract, there was significant difference in means of total worms recovered from all the treament groups (25-400 mg/kg) and also in eggs recovered from the liver and intestine tissues (50-400 mg/kg) relative to the untreated

infected (negative) control group (P<0.05) (Table 1). There was no significant difference (P>0.05) in the *E. capensis* extract treated groups when compared to the positive control (artemether treated) group.

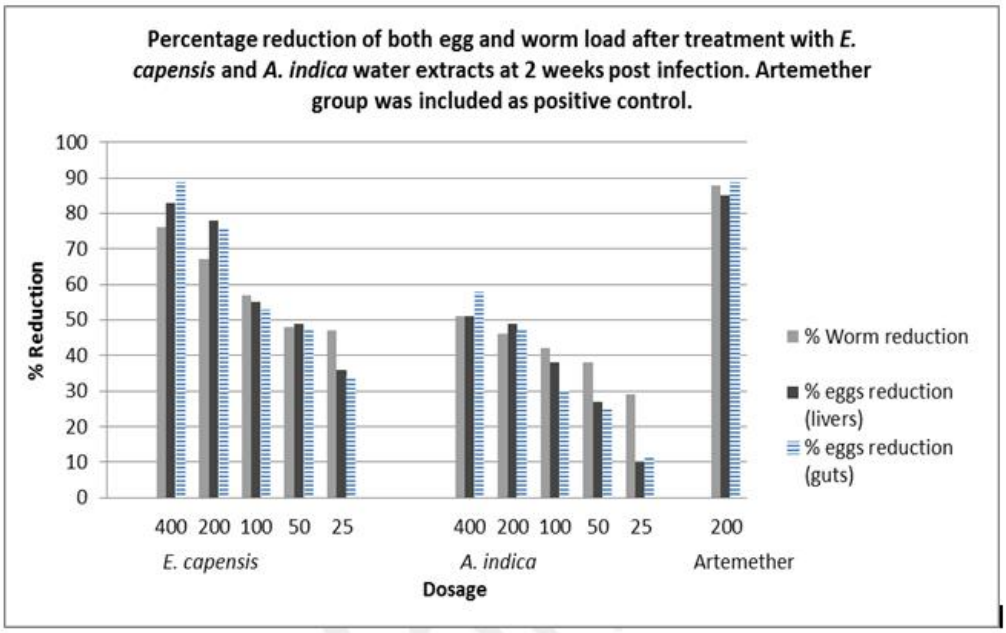

Fig. 1. Percentage worm and egg reduction after treatment with aqueous extracts of *A. indica* and *E. capensis*

Table 1. Mean number of worms and eggs recovered from livers and intestines following treatment with *A. indica* and *E. capensis* at 2 weeks post infection

Drug	Number of male worms (Mean ±SD)	Number of female worms (Mean±SD)	Total number of worms (Mean±SD)	Number of eggs (livers) (Mean±SD)	Number of eggs (intestines) (Mean±SD)
E. capensis					
400mg/kg	6.6±2.7	4.6±3	10.6±5.3[a]	2580±753[a]	1640±474[a]
200mg/kg	8.4±1.1	6±2.1	14.4±3[a]	3370±744[a]	3735±1545[a]
100mg/kg	11.2±2.3	8±2.5	19.2±4.3[a]	6900±897[a]	7284±406[a]
50mg/kg	12±3.4	11±4.8	23±7.8[a]	7772±586[a]	8176±1368
25mg/kg	14±1.6	9.6±1.1	23.6±2.6[a]	9752±934	10330±556
A. indica					
400mg/kg	10.4±2.1	11.2±3.3	21.6±4.1[a]	7350±1199[a]	6572±724[a]
200mg/kg	11.4±2.1	12.4±4	23.8±3.8[a]	7784±811[a]	8190±1097[a]
100mg/kg	12.6±2.7	13±2.1	25.6±3.8[a]	9440±854	10895±1998
50mg/kg	13.8±2.8	13.6±2.9	27.4±3.4[a]	11128±859	11697±1103
25mg/kg	15.6±3	16±2	31.6±4.8[a]	15172±1302	13709±581
Artemether	9.8±2.4	8±3.1	17.8±5.3[a]	2300±245[a]	1638±385[a]
Control	29±3.5	25±4.3	54±6.9	15307±549	15572±1342

Data are expressed as mean±SD, n=4 or 5. Data were analyzed by T-test: two sample assuming unequal variance. Values with superscript letters are significant (P<0.05), relative to untreated controls. [a] Statistically significant (P<0.05) compared to negative (untreated) controls

Similary, mice treated with *A. indica* extract showed significant difference (P<0.05) in worm load reduction in all treated groups when compared to the untreated infected (negative) control group while significant difference (P<0.05) in egg load reduction both in the liver and intestine tissues was observed at 200 mg/kg and 400 mg/kg (Table 1). When comparison was done with the artemether-treated group, there was no significant difference that was observed (P>0.05).

Artemether treated group showed significant worm load and tissues egg load reduction (P<0.05) relative to the untreated controls (Table 1).

3.2 Treatment at 4 Weeks Post Infection

When *S. mansoni* infected mice were treated at 4 weeks p.i at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg using *E. capensis* extract, more than 50% worm reduction was observed as 57%, 77% and 82% respectively (Table 2 and Fig. 2). Egg load reduction at doses of 50mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg in livers of these mice was recorded as 54%, 73%, 89% and 91%, respectively while corresponding egg load reduction from intestines of the mice at the same doses was 51%, 67%, 87% and 91% (Fig. 2). When treated using *A. indica* extract, 70% worm reduction was observed at a dose of 400 mg/kg. At doses of 50 mg/kg, 100 mg/kg, 200 mg/kg and 400mg/kg, the extract gave percentage egg reduction from the liver by 68%, 75%, 80% and 89% respectively (Fig. 2). The corresponding percentage egg load reduction from the intestines of the same mice at the same doses was 68%, 75%, 80% and 89% respectively. Artemether treated group had 60% worm load reduction, with 88% and 89% egg load reduction from the liver and intestine respectively (Fig. 2).

T-test two sample assuming unequal variance was performed (using excel data analysis tool for windows 7) to compare the statistical differences in mean worms and eggs recovered from livers and intestines between treated groups and control groups. When treated with *E. capensis* extract, all the treatment groups had significant difference (P<0.05) when compared to the untreated (negative) control in worm and egg load in both liver and intestine tissues except in mean total worm load at 25mg/kg (Table 2). When compared to the positive control (artemether) group, there was significant difference (P<0.05) in all the groups for mean worm and egg load in liver and intestine tissues except in mean worm load at 200 and 400mg/kg (Table 2).

When treated with *A. indica* extract, there was statistical difference (P<0.05) in all the groups tissues in mean worm and egg load reduction in both liver and intestine tissues relative to untreated (negative) control except for mean worm load reduction at 25 mg/kg dose group (Table 2).

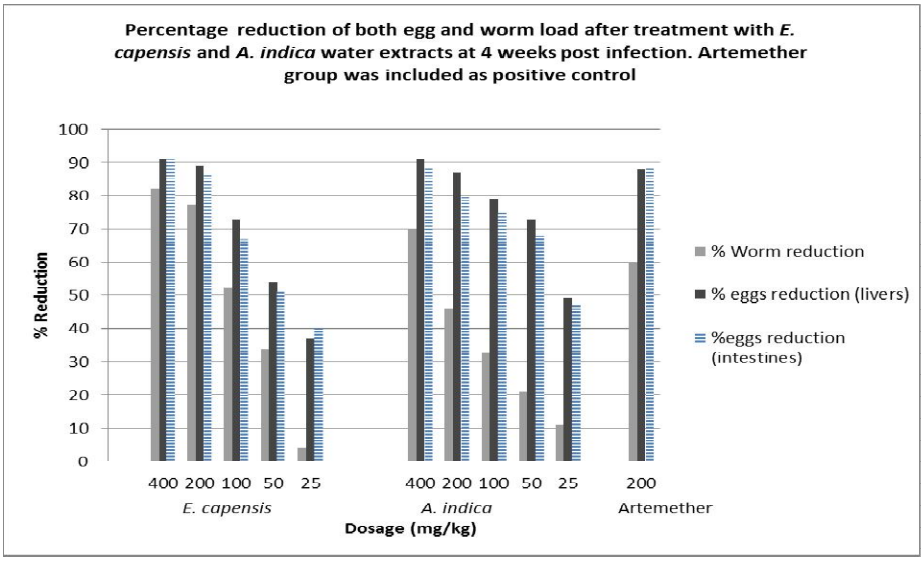

Fig. 2. Percentage worm and egg reduction after treatment with aqueous extracts of *A. indica* and *E. capensis*

Table 2. Mean number of worms and eggs recovered from livers and intestines following treatment with *A. indica* and *E. capensis* at 4 weeks post infection

Drug	Number of male worms (Mean ±SD)	Number of female worms (Mean±SD)	Total number of worms (Mean±SD)	Number of eggs (livers) (Mean±SD)	Number of eggs (intestines) (Mean±SD)
E. capensis					
400mg/kg	6.8±1.9	4.6±1.8	11.4±3.6[a]	2334±568[a]	2823±878[a]
200mg/kg	8.2±3.3	6.4±3	14.6±6.2[a]	3097±1058[a]	4001±1053[a]
100mg/kg	16.8±1.9	13±2.5	29.8±4.2[a]	7434±1150[a]	9562±871[a]
50mg/kg	22.2±3.8	19.2±6.9	41.4±10.5[a]	12552±3279[a]	15232±3680[a]
25mg/kg	30.2±2.6	30.2±2.4	60.4±3.2	17320±3439[a]	22760±7670
A. indica					
400mg/kg	9.2±5.3	9.4±5.8	18.6±10.6[a]	2475±2412[a]	3383±2404[a]
200mg/kg	18.6±1.5	15±2.2	33.6±3.5[a]	3666±1196[a]	6186±1289[a]
100mg/kg	22±2	20.2±2.58	42.2±4.2[a]	5763±1170[a]	7770±1126[a]
50mg/kg	25.4±2.6	24.8±1.3	50.2±2.8[a]	7398±1528[a]	9751±1842[a]
25mg/kg	29±3.2	26.8±4.9	55.8±6.5[a]	14007±3324[a]	16402±4265[a]
Artemether	4.6±3.4	2.2±1.5	6.8±4.8[a]	247±212[a]	446±293[a]
Control	33.4±6.9	29.2±3.7	62.6±9.1	27445±6148	30785±3654

Data are expressed as mean±SD, n=4 or 5. Data were analyzed by t-test (two sample assuming unequal variance). Values with superscript letters are significant (P<0.05). [a] Statistically significant values (P<0.05) relative to negative (untreated controls)

When the positive control (artemether) was compared with the negative control group, there was significant difference (P<0.05) in mean worm load reduction as well as liver and intestines egg load reduction as shown in Table 2.

3.3 Treatment at 7 Weeks Post Infection

When *S. mansoni* infected mice were treated with *E. capensis* extract at 7 weeks p.i at doses of 100 mg/kg, 200 mg/kg and 400 mg/kg (Table 3), percentage worm load reduction was observed as 50%, 74% and 85% respectively (Fig. 3). Percentage egg load reduction at doses of 50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg in the liver was recorded as 59%, 61%, 68% and 74% respectively, while corresponding percentage intestinal egg load reduction at the same doses was recorded as 62%, 63%, 67% and 73% (Fig. 3). When mice were treated using *A. indica* extract at doses of 200 mg/kg and 400 mg/kg (Table 3), the percentage worm reduction was observed as 51% and 55% respectively. Mice treated with *A. indica* extract at doses of 50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg had percentage egg load reduction from liver tissue of 60%, 62%, 64% and 67 while corresponding intestinal egg load reduction at the same doses was recorded as 60%, 60%, 63%, 65% and 68% (Fig. 3). The results from the PZQ treatment group were 94% worm reduction, and 99% egg load reduction for both the liver and intestinal tissues (Fig. 3).

T-test: two sample assuming unequal variances was performed (using excel data analysis tool for windows 7) to compare the statistical differences in mean worms and eggs recovered from livers and intestines between treatment groups and control groups. When treatment was done at 7 weeks post infection using *E. capensis*, all the treatment groups had significant difference (P<0.05) for both mean worm load as well as egg load reduction in liver and intestinal tissues relative to the untreated (negative) controls. There was no statistical difference that was observed in mean worm load reduction at 400 mg/kg of the plant extract relative to the positive control (PZQ treated) group (P>0.05).

When treated with *A. indica*, there was significant difference (P<0.05) in mean worm load as well as liver and intestine tissues egg load in all the treatment groups relative to infected untreated control groups.

When the positive (PZQ) group was compared to the negative (untreated) control group, significant difference (P<0.05) was observed in mean worm and egg load reduction in both liver and intestinal tissues (Table 3), an expected outcome.

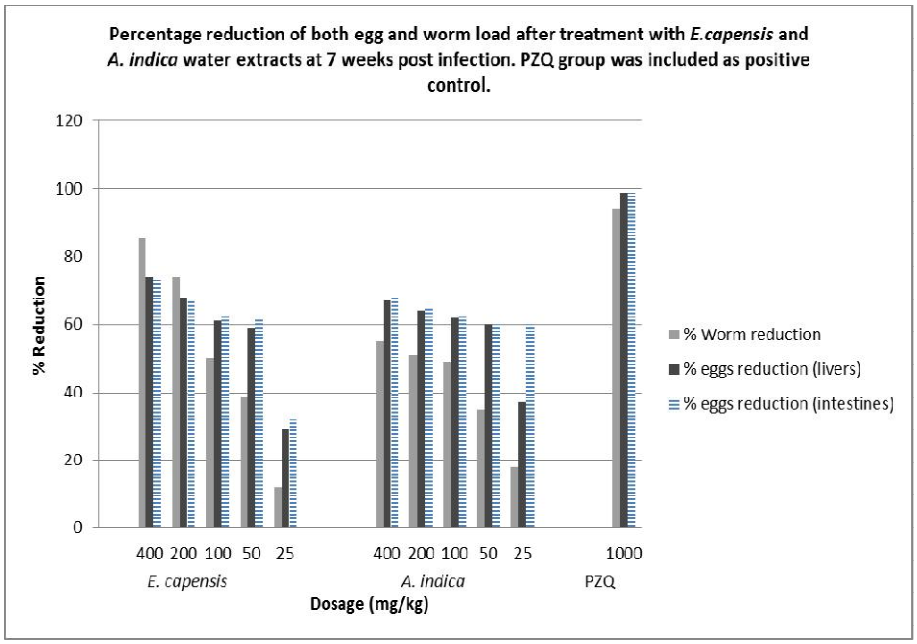

Fig. 3. Percentage worm and egg reduction after treatment with aqueous extracts of *A. indica* and *E. capensis*

Table 3. Mean number of worms and eggs recovered from livers and intestines following treatment with *E. capensis* and *A. indica* at 7 weeks post infection

Drug	Number of Male worms (Mean ±SD)	Number of Female worms (Mean±SD)	Total Number of worms (Mean±SD)	Number of eggs in the livers (Mean±SD)	Number of eggs in the intestines (Mean±SD)
E.capensis					
400mg/kg	5.8±3.7	4.8±3.3	10.6±6.9[a]	10292±1101[a,b]	11744±1604[a,b]
200mgkg	10.4±1.1	8±1.6	18.4±2.7[a,b]	12450±1766[a,b]	13428±1858[a,b]
100mg/kg	18.8±4.1	16.4±4.4	35.2±8.2[a,b]	15158±1970[a,b]	15694±1323[a,b]
50mg/kg	22.2±2.3	21.4±1.5	43.6±3.5[a,b]	15772±1371[a,b]	16110±3079[a,b]
25mg/kg	29.4±3.1	33±1.6	62.4±4.1[a,b]	27858±5442[a,b]	29298±5725[a,b]
A.indica					
400mg/kg	17±4.6	14.6±2.8	31.6±4[a,b]	12876±2643[a,b]	13603±2819[a,b]
200mg/kg	17.4±4.4	17.4±4.1	34.8±8.4[a,b]	14005±2666[a,b]	14872±3046[a,b]
100mg/kg	19±5.3	16.6±6.3	35.6±11.3	14932±3546[a,b]	15974±5229[a,b]
50mg/kg	23.8±2.4	22.4±3.6	46.2±5.4	15577±1448[a,b]	17222±1759[a,b]
25mg/kg	30.8±2.1	27.2±3.7	58±5.6[a,b]	24545±3326[a,b]	27254±3181[a,b]
PZQ	1.8±1.7	1.8±1.6	3.6±3.3[a]	278±288[a]	408±314[a]
Control	36.4±4.1	34.6±2.6	71±6.4	39142±4963	42790±4827

Data are expressed as mean±SD, n=4 or 5. Data were analyzed by t-test: two sample assuming unequal variance. Values with superscript letters are significant (P<0.05).[a]Statistically significant (P<0.05) compared to negative (untreated controls); [b]Statistically significant (P<0.05) compared to positive control (PZQ).

4. DISCUSSION

Currently there are few drugs available for the treatment of schistosomiasis these being limited to PZQ and oxamniquine and under special circumstances metrifonate and artemether may be used [19]. Of these, PZQ is the drug of choice as it is effective against all human infecting species of schistosomiasis, has the ability to irreversibly cause damage to adult worms and eggs lodged in host organ [20]. Never the less, PZQ has its limitations in that: it is less effective

against developing worms necessitating multiple drug treatments [21]; at the recommended dosage it achieves at best a 70% - 90% worm reduction and its efficacy is lowest in heavily infected individuals [22]; there are reported cases of schistosome isolates with reduced susceptibility to PZQ in the field in endemic areas and under laboratory conditions [23] which could see emergence and spread of PZQ resistant strains.

Medicinal plants may offer alternative remedies in the management of schistosomiasis and indeed there are reports of several plant species with anti-schistosomal properties [15] and [24]. Amongst these is the Chinese herb *Artemisia annua* which is effective against juvenile but not adult schistosomes [25]. The plants *A. indica* and *E. capensis* have been documented as having been used to treat a myriad of diseases that afflict man and domestic animals including tuberculosis and other bacteria, viral infections, malaria, and helminth infections [26-30]), as well as being effective against insect and tick vectors of medical importance [31]. However, there are no documented reports of evaluation of these plants for their potential anti-schistosome properties.

This study aimed at determining the antischistosomal effect of aqueous extracts of *A. indica* and *E. capensis* extracts. We have demonstrated that both of these plants have appreciable antischistosomal activity. *E. capensis* administered at a dose of 400mg/kg showed activity on both adult and juvenile worms of the parasite which was comparable to both positive control drugs (PZQ and artemether). The same was also observed in tissue egg load reduction. On the other hand, *A. indica* had low activity on adult worms which was not comparable to the activity of PZQ. It however showed a high activity on juvenile worms and this was comparable to artemether. Both aqueous extracts showed appreciable antischistomal activity but *E. capensis* was more potent with high activity on all the parasite stages. To the best of our knowledge, there is no previous reported use of *E. capensis* and *A. indica* as antischistososmal agents.

A reduction in egg load could be due to induction of separation of males and females which in turn reduces or even arrests the release of eggs, which is a relevant factor in the hepatic pathology and the transmission of the disease. A reduction in worm load especially in female worms results in a reduction in egg load in the tissues with subsequent reduction in pathology. Plant extracts with such activity have the potential of being used as transmission control tools and for intervention to ameliorate adverse effects due to disease pathology. The antischistosomal effects of these extracts on juvenile worms indicate that plant extracts may be used as complimentary drugs or in combination with conventional drugs such as PZQ for effective management of the disease.

The results from this study are supported by other studies which have reported on plants that possess antischistosomal activity. Oliveira et al. [32] reported that *Baccharis trimera* Less DC "Carqueja-amarga" exhibited schistosomicidal effect *in vivo* against immature, adult worms of *S. mansoni* and also significant reduction in egg load due to reduction in worm load. The crushed seeds of the plant *Nigella sativa* have also been found to have antischistosomal activity against different stages (cercariae and juvenile worms) of *S. mansoniin vitro* [33]. These results are also in harmony with schistosomicidal activity of crude aqueous extract of ginger against *S. mansoni* reported by Mostafa et al. [34] who observed that parasite load and egg density in the liver and faeces of mice treated with ginger were smaller than their counterparts. Male worms recovered from mice treated with ginger lost their normal surface architecture, extended erosion beyond the tegument, besides numerous bubbles around tubers. Mahmoud et al. [33] reported that treatment of mice infected with *S. mansoni* parasite using black seed oil, was effective in reducing egg count in both liver and intestine.

5. CONCLUSION

In conclusion, *E. capensis* and *A. indica* aqueous extracts demonstrated reduction of *S. mansoni* infection at different developmental stages. *E. capensis* showed greater ability in worm burden reduction and also tissue egg load reduction. Further studies should be done to determine if the extracts from the plants can be used singly or in combination with PZQ in the management of schistosomiasis since this could become a strategy for transmission control which can reduce the morbidity of schistosomiasis in endemic regions. Isolation and characterization of the active compound(s) of these plants and determination of their mechanism(s) of action is also recommended in search for novel antischistosomal agents.

CONSENT

Not applicable.

ETHICAL APPROVAL

The mice used in this study were maintained according to international accepted procedures for animal care and management as recommended by the Kenya Medical Research Institute Animal Care and Use Committee (ACUC). All the mice in this experiment were maintained in clean well labelled cages and their beddings changed twice a week. These mice were maintained on water and mice pellets *ad libitum*. These mice were handled humanely at all times by anaesthetizing them during infection and perfusion using pentobarbitone sodium. After perfusion, the mice were disposed in the incinerator in biohazard plastic bags at the end of the study.

All authors hereby declare that "principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee. The approval for conducting this study was obtained from the Scientific Steering Committee (SSC) and the Ethical Review Committee (ERC) protocol number SSC 2526 from Kenya Medical Research Institute.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Carter Center. Schistosomiasis Control Program; 2008. Available:http://www.cartercenter.org/health/schistosomiasis/index.html
2. World Health Organisation. Schistosomiasis fact sheet; 2010. Available:http://www.who.int/mediacentre/factsheets/fs115/en/index.html Accessed 2012 Aug 30
3. World health organization. Schistosomiasis and soil transmitted helminths country profile. 2006. World health assembly resolution 54.
4. Cioli D, Pica-mattoccia I. Praziquantel. Parasitol res. 2003;90(Supp 1):3-9.
5. Gönnert R, Andrews P. Praziquantel, a new Broad-spectrum antischistosomal agent. Zeitschrift für parasitenkunde (berlin, germany). 1977;52(2):129-150.
6. Pica-mattoccia I, Ruppel A, Xia CM and Cioli D. Praziquantel and the benzodiazepine ro 11-3128 do not compete for the same binding sites in schistosomes. Parasitol. 2007;135:47-54.
7. Doenhoff MJ, Cioli D, Utzinger J. Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. Curr Opin Infect Dis. 2008;21:659-667
8. El-lakkany NM, El-din SHS, Sabra ANAA, Hammam OA. Pharmacodynamics of mefloquine and praziquantel combination therapy in mice harbouring juvenile and adult *Schistosoma mansoni*. Meminstos waldocruz. 2011;106:814-822.
9. Berquist NR, Sherbiny ACM, Barahat R, Olds R. Blueprint for schistosomiasis vaccine development. Acta trop. 2002;82:183-192.
10. Ross APG, Bartley PB, Sleigh AC, Olds GR, Li Y, Williams GM, et al. Schistosomiasis. N Engl J Med. 2002;346: 16.
11. Brindley PJ, Strand M, Norden A, Sher A. A role of host antibody in the chemotherapeutic action of praziquantel against *Schistosoma mansoni*. Molbiochemparasitol. 1989;34:99-108.
12. Kokwaro JO. Medicinal plants of East Africa. 1993; Second edition, Kenya Literature Bureau, Nairobi, Kenya.
13. Ndamba J, Nyazema N, Makaza N, Anderson C, Kaondera KC. Traditional herbal remedies used for the treatment of urinary schistosomiasis in zimbabwe. J. Ethnopharmacol. 1994;42:125-132.
14. Sheir Z, Nars A, Massoud A, Salam O, Badra GA, El- Shennay H, et al. A safe, effective herbal antischistosomal therapy derived from myrrh. Am. J. Trop. Med. Hyg. 2001;65(6):700-704.
15. Sparg SR, Van Staden J, Jager AK. Efficiency of traditionally used South African plants against schistosomiasis. J. Ethnopharmacol. 2000;73(1-2):209-214.
16. Xiao SH, Chollet J, Weiss NA, Bergquist RN and Tanner M. Preventive effect of artemether in experimental animals infected with *Schistosoma mansoni*. Parasitol. Int. 2000;49:19-24.
17. Smithers SR and Terry RJ. The infection of laboratory hosts with cercariae of

Schistosoma mansoni and recovery of the adult worms. Parasitol. 1965;55:695.
18. Utzinger J, Chollet J, Tu ZW, Xiao SH, Tanner M. Comparative study of the effects of artemether and artesunate on juvenile and adult *Schistosoma mansoni* in experimentally infected mice. Trans. R. Soc. Trop. Med. Hyg. 2002;96:318-323.
19. Doenhoff M, Cioli D and Kimani, G. Praziquantel and the control of schistosomiasis. Parasitol. Today. 2000;16:364-366.
20. Giboda M, Smith JM. *Schistosoma mansoni* eggs as a target for praziquantel: efficacy of oral application in mice. J. Trop. Med. Hyg. 1994;97:98-102.
21. Utzinger J, Keiser J, Shuhua X, Tanner M, Singer BH. Combination chemotherapy of schistosomiasis in laboratory studies and clinical trials. Antimicrob Agents Chemother. 2003;47:1487-1495.
22. Raso G, N'goran EK, Toty A, Luginbühl A, Adjoua CA, Tian-bi NT, et al. Efficacy and side effects of praziquantel against *Schistosoma mansoni* in a community of Western Côte d'Ivoire. Trans R Soc Trop Med Hyg. 2004;98(1):18-27.
23. You-Sheng L, Jian-rong D, An N, Dong-bao Y, Xing-jian X, Yin-chang Z, et al. Susceptibility of *Schistosoma japonicum* to praziquantel in China. Trop Med Int. 2001;6:707-714.
24. Molgaard P, Nielsen SB, Rasmussen DE, Drummond RB, Makaza N, Andreassen J. Anthelmintic screening of Zimbabwean plants traditionally used against schistosomiasis. J Ethnopharmacol. 2001; 74:257-264.
25. Utzinger J, Xiao SH, N'goran EK, Bergquist R, Tanner M. The potential of artemether for the control of schistosomiasis. Int. J. Parasitol. 2001;31:1549-1562.
26. Elavarasu S, Abinaya P, Elanchezhiyan S, Thangakumaran, Vennila K, Naziya KB. Evaluation of anti-plaque microbial activity of *Azadirachta indica* (neem oil) *in vitro*: a pilot study. J Pharm Bioallied Sci. 2012;4(Suppl 2):s394-6.
27. Muregi FW, Chhabra SC, Njagi EN, Lang'at-Thoruwa CC, Njue WM, Orago AS. Anti-plasmodial activity of some Kenyan medicinal plant extracts singly and in combination with chloroquine. Phytother res. 2004;18(5):379-384.
28. Lall N, Meyer, JJM. *In vitro* inhibition of drug-resistant and drug-sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South African plants. J Ethnopharmacol. 1999;8:347-354.
29. Chandrawathani P, Chang KW, Nurulaini R, Waller PJ, Adnan M, Zaini CM. Daily feeding of fresh neem leaves (*Azadirachta indica*) for worm control in sheep. Trop biomed. 2006;23(1):23-30.
30. Abakala M, Oyewole OA, Kolawole AR. Antibacterial activities of *Azadirachta indica* against some bacterial pathogens. Adv life Scie. 2012;2(2):5-8.
31. George DR, Finn RD, Graham KM, Sparagano OAE. Present and future potential of plant-derived products to control arthropods of veterinary and medical significance. Parasit Vectors. 2014;7:28.
32. de Oliveira RN, Lúcia V, Rehder G, Silva A, Oliveira S, de Lourdes V, et al. Anthelmintic activity *in vitro* and *in vivo* of *Baccharis trimera* (Less) DC against immature and adult worms of *Schistosoma mansoni.* Exp. Parasitol; 2014. Article in Press.
33. Mahmoud MR, El-Abhar HS, Salesh S. The effect of *Nigella sativa* oil against the liver damage induced *Schistosoma mansoni* infection in mice. J Ethnopharmacol. 2002;79:1-11. ISSN: 0378-8741.
34. Mostafa OM, Eid RA, Adly MA. Antischistosomal activity of ginger (*Zingiber officinale*) against *Schistosoma mansoni* harbored in C57 mice. Parasitol. Res. 2011;109:395-403.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=903&id=13&aid=7640