Research Article

In Vitro and in vivo Efficacy of Three Medicinal Plants on adult worms of Schistosoma mansoni

Saada Mohamed Nour*[1], Yahia Fadul Tahir [2], Ikram Madanin Ahmed [3] and Mona A M Mageed [4]

[1]Department of Microbiology, College of Medicine, University of Bahri, Khartoum, Sudan.
[2]Department of Archeology, Faculty of Art, University of Khartoum, Khartoum, Sudan.
[3]Department of Botany, Faculty of Science, University of Khartoum, Khartoum, Sudan.
[4] Department of Pharmacognosy, Faculty of Pharmacy, Omdurman Islamic University, Omdurman, Sudan

*Correspondence: Saada Mohamed Nour Kheiry, Tel. 002490122547151, email saada.nori@yahoo.com

Abstract

Background: Schistosomiasis is a common disease in the tropics and subtropics. Although praziquantel (PZQ) is the drug of choice due to its high cure rates, the development of PZQ resistance by different Schistosoma species has been observed.

Aim: This study was an attempt to find an alternative to PZQ by evaluating the activity of the raw aqueous extracts of three medicinal plants: Hagenia abyssinica (fruits), Ambrosia maritima L. (leaves) and Catunaregam atunaregam nilotica (fruit), against adult worms of Schistosoma mansoni.

Methods: In vitro, the extracts were used against adult worms in different concentrations (10000, 5000, 1000, 500, 250, 50, and 25 ppm) compared to the same concentrations of PZQ. The time of worm paralysis and worm death was determined. For the in vivo study, infected mice were divided into three groups i untreated ii PZQ-treated at dose (40 mg/kg) iii aqueous raw extracts treated at dose (1 ml of 10 000 ppm).

Results: All adult worms exposed to Catunaregam nilotica (50ppm) in vitro, died within 20 to 25 minutes after incubation, while Hagenia abyssinica (250ppm) required 1-1.5 hours to kill all adult worms, but Ambrosiamaritima (1000 ppm) killed all adult worms within 3 hours. Mice treated with aqueous extracts of C. nilotica and praziquantel looked very healthy. The percentages of total worm burden reduction in mice treated with the aqueous extracts: C. nilotica = 55%, H. abyssinica =40.2%, A. martima = 24.2%, and praziquantel = 59%.

Conclusion: The metabolites of Catunaregam nilotica (fruit) were the most potent schistosomicidal which revealed the lowest toxic concentration that killed all worms within the shortest time. Hagenia abyssinica (fruit) and Ambrosia maritima L. (leaves) were also safe and promising metabolites for the treatment of schistosomiasis.

Keywords

Catunaregam nilotica, Hagenia abyssinica, Ambrosia maritima, Schistosoma mansoni

Introduction

Schistosomiasis is an important public health problem in rural communities located near slow-moving water canals in the tropical and sub-tropical regions with an effect on the development of socio-economic state of affected area. Schistosomiasis represent the second parasitic disease in the world after malaria, considering the number of people infected and the extent of endemic areas. Chemotherapy is the main strategy of control, with praziquantel the drug of choice for treatment of scistosomiasis (WHO, 1999)(Sakina and Ahmed, 2018). Because of the tolerance or resistance to praziquantel, research to produce new drugs for the cure and prevention of *Schistosoma mansoni* has become realistic. Ismail, et. Al. (1994) and (Abdel Aziz *et. al*., (2018) reported that the use of PZQ., especially in low sub curative dose may cause the resistance of the therapeutic dose of the drug in the coming generations. A large number of Sudanese endogenous plants are reported as a sources for the control of snail hosts and chemotherapy of schistosomiasis (Suliman and Ebrahim, 1994). This study was aimed to evaluate the effect of aqueous extracts of three medicinal plants used in Sudanese folk medicine (fruits of *Hagenia abyssinica* (*Bruce* ex *Steud*.) J.F. *Gmel*, leaves of *Ambrosiamaritima* L.), and fruit part of *Catunaregam nilotica* (*Stapf*) *Tirveng*. On adult male and female worms of *S. mansoniin vitro* and *in vivo*.

Materials and Methods

Plant material

Catunaregam nilotica (fruits) were collected from western Sudan (Kordofan province) and eastern Sudan (Angesna Mountains). *Ambrosia maritima* leaves) were collected from northern Sudan (Northern Province, Maha's area). *Hagenia abyssinica* (fruits) were purchased from herbalisms in Khartoum state. All plant samples were authenticated by Dr. Ikram Madanin Ahmed, Department of Botany, Faculty of Science, University of Khartoum, Khartoum, Sudan

Preparation of extracts

Dry clean coarse plant material was macerated with distilled water and kept for 24 hours at room temperature. The extract was filtrated and dried using a rotary evaporator (Elshafei, Habiballa, and Mohamed, 2019). Ten grams of the dry extract was dissolved in 1000ml distilled water to give a solution of 10 g /liter (10000 ppm). Further serial dilutions were prepared using the above stock solution to give concentrations of 5000 1000, 500, 250, 100, 50, and 25ppm (de Oliveira *et al*., 2017).

Praziquantel which was obtained from (Ministry of Health, Department of schistosomiasis) was freshly suspended in 13ml of 2% Cremophore (El Sigma Chemical Co.) and orally administered to mice at a dose of 40mg/kg bodyweight single dose(Al Hamshary *et al*., 2018).

Production of Miracidia

Stools were collected from school children of 10-15 years of age in the area of agricultural schemes situated north of Elgezira state (Elsiraha village). The stools were examined for the presence of *S. mansoni* eggs. Positive stools were processed, hatched, and miracidia were collected in a clean Petri- dish and counted under a dissecting microscope (Al Hamshary *et al.*, 2018).

Preparation of Snails

Biomphalaria pfeifferi snails were collected from the irrigation canals around Khartoum, from different localities in Elsilate and Kriab agricultural schemes. The snails were washed with filtered aquarium water to remove contaminants from the field and each batch was examined three times over one month to ensure their freedom from infection. Part of snails was reared until thy gave the juvenile snails which were transferred to ordinary maintenance aquaria after the age of four weeks (Al Hamshary *et al.*, 2018).

Production of cercariae and infection of snails:

Groups of 25-30 infected (*Biomphalaria sp*) snails were placed in 50 ml beakers and washed twice with water to remove fecal and food particles. Warm water at 40°C was added to the snails and placed under a source of light for 1-2 hours for shedding cercariae (Al Hamshary *et al.*, 2018).

Batches of snails were exposed singly to infection with 3-4 miracidia of *S. mansoni* overnight, then transferred to bowls containing water and maintained at room temperature until the shedding of cercariae (Al Hamshary *et al.*, 2018).

Preparation of adults worms of *Schistosoma mansoni*:

White albino mice, weighing 18-25 g were infected by the whole body method as described by of Webbe and Jemmes (1971). using 100 cercariae. Mature Schistosoma worms recovery was assessed by portomesenteric perfusion technique 8 -10 weeks after infection, according to the method of Duvall and De Witt (Al Hamshary *et al.*, 2018). Adult worms were collected in normal saline.

Evaluation of plants extracts efficacy

In vitro toxicity to adult worms

Ten worms (5 pairs) were transfered into each well of the microtitre plates. 1 ml of different concentrations of each extract was added to each well of the microtitre plates containing worms.

In vivo toxicity to adult worms

A total of 60 male and female white albino mice of 20-25 gm weight, without any entero parasitic infection were, divided into 6 groups with 10 mice in each group. Five of these groups were infected with 100 cercariae of *S. mansoni* for each mouse by the whole body method (Webbe and Jemmes, 1971). Six weeks post-infection the eggs of *S. mansoni* appeared in the feces of mice. Three groups of mice were given with 1 ml of 10 000 ppm of aqueous extract of *C. nilotica*, *H. abyssinica*, and *A. martima* orally daily for 1 week. The 4th group was given 1

dose of Praziquantel 40 mg /kg body weight (Table1). The 5th group was equally infected but not treated and were kept as infected control, finally, the 6th group of 10 mice was not infected and not treated but was kept as a healthy control group.

Experiments were performed in 5 replicates. Clinical signs, postmortem lesions, the mean egg counts / mouse, and total worm burden and female worm burden of all groups of mice were observed and calculated after 8 weeks (Hassanzadeh *et al.*, 2019).

Table (1): Aqueous extracts and Praziquantel administration schedule via oral route to the mice infected with *S.mansoni*.

Group No.	Extract/Drug	No. of mice/ group	Dose	Days of administration (after infection)
G 1	Catunaregam nilotica	10	1 ml of 10 000 ppm	7
G 2	Hagenia abyssinica	10		7
G 3	Ambrosia martima	10		7
G 4	Praziquantel	10	40 mg/kg	1
G 5	infected control	10	Not treated	
G 6	Free of infection	10	Not treated	

Result

In vitro toxicity to adult worms

C. nilotica aqueous extract was the most potent schistosomicidal (Table 2) which revealed the lowest toxic concentration that killed all worms within the shortest time.

Table (2): *In vitro* wormicidal activity of the aqueous extracts of the three plants on adult worms of *Schistosoma mansoni*.

Plant	Minimum toxic concentration (ppm)	No. of worm pairs	Time of death
Catunaregam nilotica	50	5	20-25 minutes
Hagenia abyssinica	250	5	1-1.5 hours
Ambrosia maritima	1000	5	3 hours

In vivo toxicity to adult worms

Clinical signs observed

Clinical signs that were observed of infected untreated mice after 8 weeks were as follows:

1. Severe itching during the first 2 weeks in the legs and neck and all the body after 6 weeks
2. Harsh coat in the neck part in the first 3 weeks and all the body after 6 weeks.
3. Change in the color of hair to pale.
4. Rectum prolapse and enlarged abdominal cavity.
5. Inappetanceand dullness.
6. Enlargement in the posterior part of the abdomen.

After 10 weeks most of the signs disappeared and they became well. All normal, not infected, and not treated mice were healthy.

Postmortem lesions of infected untreated mice after 8 weeks of infection were:

1. Enlarged mesenteric tissue (facia).

2. Enlarged, inflammed, and hemorrhagic liver with biogenic foci.
3. Congested mesenteric blood vessels.
4. Empty intestine.

Clinical signs and post mortem findings of mice treated with aqueous extracts were as follows:

All mice treated with *A. martima* and *H. abyssinica* showed clinical signs as those in the control group(Infected Untreated Mice). The mice treated with aqueous extracts of *C. nilotica* and Praziquantel looked very healthy and alert, with smooth hair and clear color after dosing.

Post mortem findings of those mice treated with *A. martima* and *H. abyssinica* were typical as those in the control group. Minor lesions were found in those treated with aqueous extract of *C. nilotica*, enlarged liver and slight congestion of mesenteric blood vessels. Slight enlargement of the liver was observed in those treated with Praziquantel.

The mean egg counts in the liver and intestine per infected untreated mouse were 54.801. It decreased in those treated with Praziquantel and aqueous extract of *C. nilotica* to 29.240 and 14.127 respectively. The mean egg counts in mice treated with *H. abyssinica* and *A. martima* were 51.225 and 56.139 respectively (Table 3).

The percentages of total worm burden reduction in mice treated with the aqueous extracts of *C. nilotica* was 55 %, *H. abyssinica*, 40.2 %, *A. martima* 24.2 %. Praziquantel gave 59% (Table 3).

Table .3. The percentages of total worm burden reduction in mice treated with the aqueous extracts of *Catunaregam. nilotica, Hagenia abyssinica, Ambrosia martima,* and Praziquantel.

Group No.	Plants / drug	Mean ±S.D total worm burden	Worm burden reduction (%)	Mean ± S.D female worm burden	Female worm burden reduction (%)
G 5	Infected control	29.88 ± 14.5	-	13.87 ± 5.7	-
G 1	*Catunaregam nilotica*	13.44 ± 4.3 ***	55.02	6.78 ± 2.6 ***	51.2
G 2	*Hagenia abyssinica*	17.88 ± 5.9 *	40.16	9.77 ± 3.2	29.5
G 3	*Ambrosia maritima*	22.66 ± 8.1 n.s	24.16	11.88 ± 4.3 n.s	14.5
G 4	Praziquantel	12.25 ± 4.6 ***	59	3.87 ± 2.0 ***	72.1

Key: *** highly significant P<0.005 .* moderate significant n.s (not significance) s.d (stander deviation)

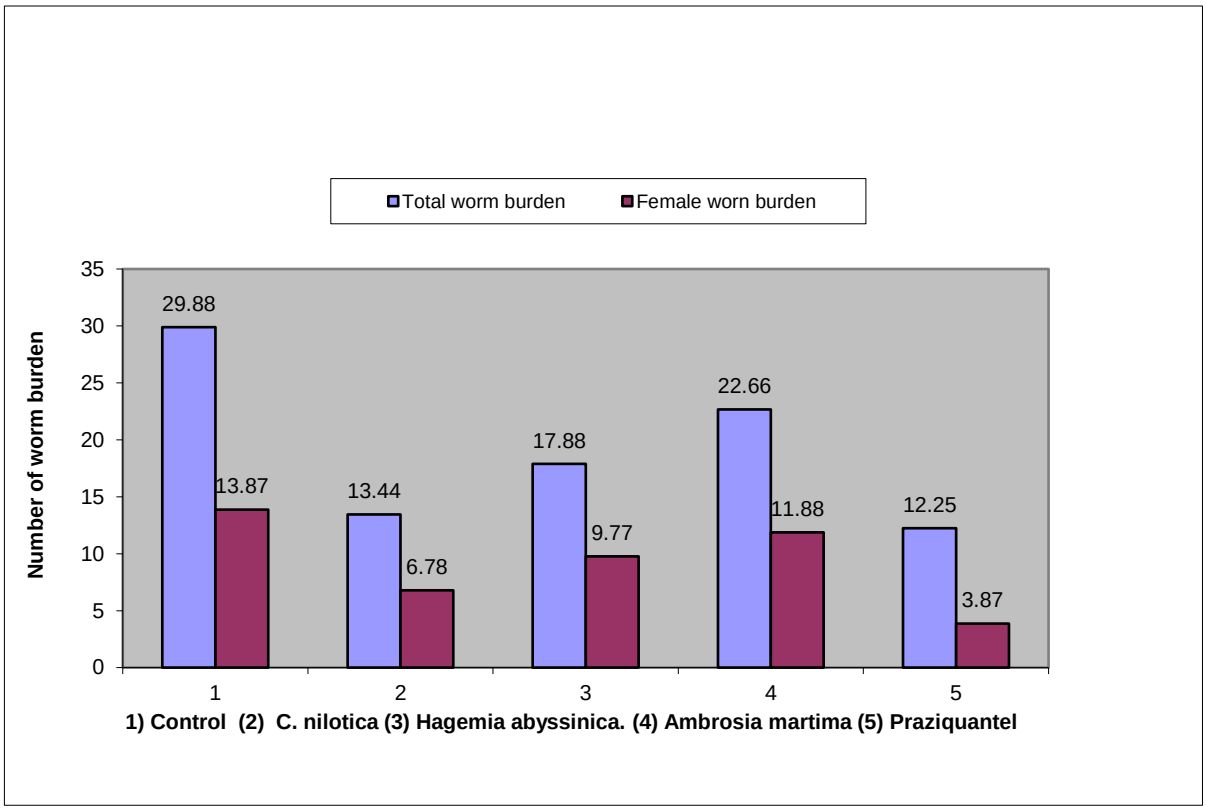

Fig. (1). Histogram of mean total and female worm burden in mice treated with the 3 plant extracts and Praziquantel.

Discussion:

The aqueous extracts of the two medicinal plants (*H. abyssinica* and *A. maritima*) used in these experiments against adult *S. mansoni* showed lower actions in comparison to *C. nilotica* and this was clear in the duration of time taken for the killing of worms and the changes in their shapes. The weak effect of *A. maritima* against *S. mansoni,* when given orally, is consistent with Abodome (1994); Pereira *et al* (2018); Hassanzadeh *et al* (2019) and Acheampong *et al* (2020)who reported that administration of *A. maritima* orally has insignificant effect on *S. mansoni* in mice. Saada (2005), Aladdin, *et al*, (2009) Mwangi *et al.* (2017); Abdel Aziz *et al.*, (2018); Issa *et al.*, (2018) indicated the therapeutic effect of *C. randia* against *S. mansoni*. The lethal concentration of aqueous extract of *C. nilotica* that killed adult worms of *S. mansoni* agreed with EL Sheikh findings (1994), Murugeswaran *et al*.(2016); Tekwu *et al.*(2017); Pollution and Health (2018); Mwagandi Chimbevo and Essuman (2019) and Hassanzadeh *et al.* (2019). The results of *in vivo* studies by aqueous extracts of the three plants: *C. nilotica, A. martima, and H. abyssinica,*in comparison with Praziquantel-the drug of choice in schistosomiasis treatment (WHO 1999)-showed that the aqueous extract of *C. nilotica* was more toxic to the adult *S. mansoni* ($P<0.005$) than the two other plants. It resulted in 55 % of the total worm-burden reduction and 51% of female worm-burden reduction while Praziquantel resulted in 59% in total worm burden reduction and 72. % in female worm-burden reduction in comparison to the control group. Saada (2005) stated that methanol extract of C. *nilotica*at concentration of 1000 ppm resulted in 70% of the total worm burden reduction and 96% in female worm-burden reduction. Egg counts of the liver and the intestines decreased more in mice treated with C. *nilotica* aqueous extract than those treated with Praziquantel and the other plant extract[s] . This agree with (Romero-Benavides *et al.*, 2017; and (Dias *et al.*, 2019). This means that *C. nilotica* is also toxic to the eggs of *S. mansoni.* The size and the shape of worms collected from mice treated with *C. nilotica* extract were very small and thread-like and they were inactive but the worms recovered from Praziquantel treated mice were normal and were active and this agrees with (Murugeswaran *et al.*, 2016). The appearance of mice was, smooth, alert, and healthy. The size of the liver in both groups of mice treated with Praziquantel and C. *nilotica* extract returned to normal, This agrees with the findings of (Quílez *et al.*, 2018).

Conclusion

It was concluded that the aqueous extract of *C. nilotca* is a very effective antischistosomal agent. And this will participate in the current research for alternative therapy for Praziquantel-resistant strains.

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