

Efficacy of aqueous and methanol extracts of *Randia nilotica* against Migrating juvenile of *Schistosoma mansoni* in comparison with Praziquantel in Mice

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Abstract

Introduction:

Schistosomiasis is an important parasitic disease in the tropics. Praziquantel is unable to treat the migrating juvenile which stressed the need for new drugs.

Objective:

To evaluate the effectiveness of a plant (*Randia nilotica*) used traditionally to treat migrating juvenile form of *schistosoma mansoni*.

Methods:

Sets of experiments were carried out using both aqueous and methanol extracts of *R. nilotica* and Praziquantel drug to evaluate their efficacy against migrating schistosomulae by different routes of administration and regimens. First group given was 1 ml of 10 000 ppm of aqueous extract orally and intrapratonially from day 2-8 post infection. Second group was given three doses of 1 ml of 500 ppm of methanol extract on day 7, 21 and 35 after infection. The third group of mice was given 7 doses of Praziquantel at 60 mg /kg orally from day 2-8 post infection.

Results:

It resulted in significant reduction in total worm burden at 50% and 73% respectively. The size of worms was very small. Obvious reduction result in mean eggs counts in liver and intestine per mouse in first group.

The total worm burden reduction was 83% but the worms were normal, very active (high motility rate) and no dead worms in second group. The third group of mice, showed non significant reduction in total worm burden which was (29%).The worms were normal, very active.

Conclusion:

We concluded that the aqueous extract of *R. nilotica* is highly effective against immature worms of *S. mansoni*.

Keywords: worms burden, intrapretonial, cirrhosis.

Schistosomiasis remains an important parasitic disease, widely distributed through tropical and subtropical countries. It continues to occupy the second position in the world among parasitic diseases

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after malaria, in terms of the extent of endemic areas and the number of people infected. The mainstay of control is chemotherapy, with Praziquantel being used as the drug of choice¹ but unfortunately it is less active against juvenile stages of *Schistosoma mansoni* than the adult schistosomes²⁻³.

Consequently our interest geared towards discovering a new schistosomocidal agent against schistosomulae from medicinal plants that are used in folk medicine.

Based on results in our previous studies, *Randia nilotica* plant (which is traditionally used to treat jaundice) showed a high efficacy

against adult worms of *S. mansoni*. Methanol extract of *Randia nilotica* when given to mice infected with *Schistosoma mansoni* resulted in 87% inhibition of worm burden as compared with the control mice. Praziquantel when given to infected mice under the same conditions resulted in 59% inhibition of total worm burden⁴. Accordingly, it was decided to investigate its efficacy against migrating juvenile *Schistosoma mansoni*.

Objectives: To investigate therapy with *Randia nilotica* against migrating juvenile *Schistosoma mansoni*.

Plan of the experiments:

Experiment 1:

In this section the experiments were designed to study the efficacy of aqueous extract of *R. nilotica* against migrating juvenile of *Schistosoma mansoni* (*in vivo*) in white albino mice recently infected (Dosing was in first week after infection from day 2 to day 8).

Experiment 2:

In this section the experiments were designed to study:

The efficacy of Methanol extract of *R. nilotica* against migrating juvenile of *Schistosoma mansoni* (*in vivo*) in white albino mice (dosing was given at intervals of 7, 14, 28. days after infection).

Experiment 3:

In this section the experiments were designed to study the efficacy of praziquantel against migrating juvenile of *Schistosoma mansoni* (*in vivo*) in white albino mice recently infected (Dosing was in first week after infection from day 2 to day 8).

Materials and methods

Plant material:

Fruits of *Randia nilotica* were collected from Eastern and Western Sudan (Angešana and Nubian Mountains), where it is used in traditional medicine for treatment of jaundice. The aqueous extract was prepared by macerating 10 gram of coarsely powdered fruit part in 800 ml of distilled water for 24 hours at room temperature. The extract solution was filtered and adjusted to 1000 ml with distilled water to give a solution of 10 g / litre (10000 ppm). The methanol extract was prepared by mixing 350 grams of coarsely

powdered fruit part with 99% methanol for 24 hr using a soxhlet apparatus.

Parasitological techniques:

Schistosoma mansoni cercariae were obtained by infecting *Biomphalaria pfefferi* snails collected from Elkryab village (North of Khartoum) with miracidia obtained from eggs collected from feces of school children infected with *S. mansoni* at Elsiraha village, Gezira Province, Sudan. Cercariae obtained after 4 weeks after snail infection

Mice were infected by cercariae using paddling method of Webbe⁵.

The 10 mice, of the 4th control group and all treated groups, were sacrificed, perfused and all worms were collected, and differentiated into males and females and counted after 8 weeks post infection and treatment by the perfusion techniques described before⁶. Control group was scarified, observing the standard animal rights legislations.

Drug:

Praziquantel was employed for comparison. It was obtained from the ministry of health, Departement of Schistosomiasis control (Sudan).

The animals:

Sixty white albino mice, of both sexes and of 20-25 gm. weight, were used in this study. These mice were bred and maintained on the regular laboratory diet, in the faculty of pharmacy of Khartoum University where the study was done. The experimental design and the administration schedule are described in Table 1.

Egg counts:

Fecal eggs were detected by the standard method⁷. The eggs of tissues (liver and intestine) from perfused animals were counted by the KOH digestion method⁸.

Post mortem lesions:

Mice were scarified for worm retrieval and all observed lesions were recorded.

Statistical analysis:

The effects of *R. nilotica* and Praziquantel in this experiment were assessed by comparing the mean number of total (and female) worms in any of the treated groups with the control group. For statistical analysis, student t-test was used.

Table (1): Aqueous and methanol extracts of *Randia nilotica* and Praziquantel administration schedule for the mice infected with Juvenile schistosomulae.

Groups	Extract/Drug	No.of mice	Route of Administration	Dose	No.of cercariae	Days of administration
G1 a	Aqueous extract of <i>R. nilotica</i>	10	Oral	1 ml of 10 000 ppm	150	2-8 a .i
G1 b	Aqueous extract of <i>R. nilotica</i>	10	intrapretonial	1 ml of 10 000 ppm	150	2-8 a .i
G 2	Methanol extract of <i>R. nilotica</i>	10	intrapretonial	1 ml of 500 ppm	150	7, 21, 35 a .i
G 3	Praziquantel	10	Oral	60 mg/kg	150	2-8 a .i
G 4	Control (infected untreated)	10	-	-	150	-
G 5	Free of infection	10	-	-	-	-

Results:

Results of total worm burden and female worm burden reductions:

Single daily intrapretonial (i.p) dose of 1ml of plant methanol extrat at 500 ppm on days 7, 21, 35, resulted in a significant total-worm burden reduction of 83% and female worm burden reduction of 81% but the recovered worms were normal in size and were active. On the other hand, the oral administration of 7 doses of 1 ml of 10 000 ppm of the aqueous extract of the plant from day 2 to day 8 after infection, resulted in total worm burden reduction of 50% and female worm burden reduction of 41% and those administered intrapretonially at the same time from day 2 to 8 with the same doses and concentration, resulted in total worm burden reduction of 73% and female worm burden reduction of 72%. But most of the recovered worms were dead and the alive ones were minute (stunted growth) and not active. (Plate1).

The stunted worms were studied histologically (plate 2) the spines were small, few and oval in shape. The cuticle and muscular layers were not clearly distinguished. The nuclei were not clear.

The group of mice treated orally with Paraziqantel by 7 doses of 60 mg/ kg from day 2 to day 8 resulted in total worm burden reduction of 29% and female worm burden reduction of 14 %. The recovered worms were normal and active as those recovered from control group.



Plate. 1. Stunted *S. mansoni* worm collected from mice treated with aqueous extract of *R. nilotica* in first week of infection x 4.



Plate: 2. A section of *S. mansoni* worm collected from mice treated with methanol extract of *R. nilotica* in first week of infection. X200

Fig. 1 showed the results of total worm burden and female worm burden reductions.

Clinical Signs and postmortem Lesions of Infected and Untreated Mice After 8 Weeks:

The control group, infected untreated mice, showed typical signs and postmortem lesions of mansonial schistosomiasis.

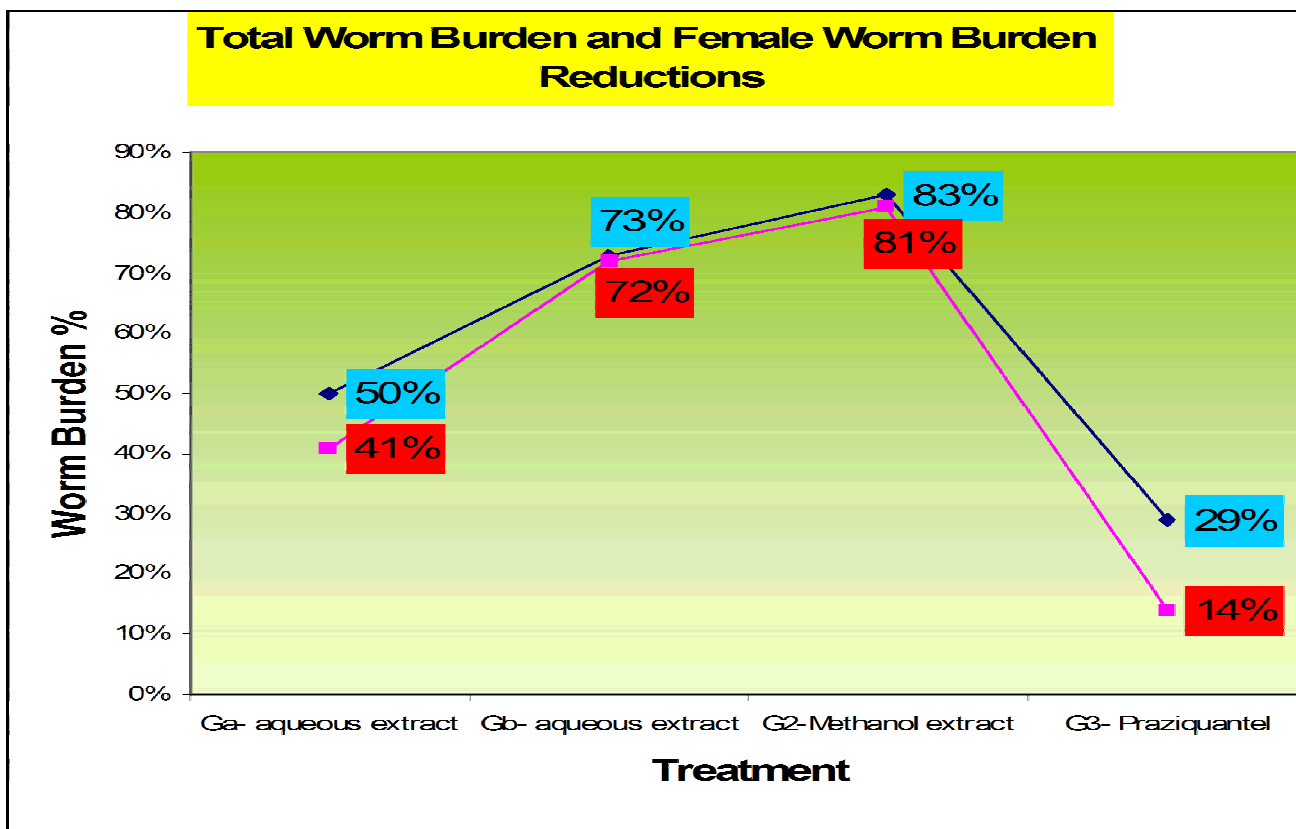


Fig. 1. Results of total worm burden and female worm burden reductions.

Table 2: The clinical signs and post mortum lesions of mice in different treated groups:

Groups of mice	Clinical signs	Post mortum after 8 weeks
G1 a	minor itching first 2, 3 days	No lesion
G1 b	minor itching first 2, 3 days	No lesion
G 2	Minor itching first week	No lesion
G3	Showed most of the signs as in the control group	Showed most of the lesions as in the control group

G= group

The clinical signs and post mortum lesions of mice in different treated groups is summarized in table 2.

The mean egg count per mouse in different prophylactically treated groups:

The mean egg counts in the liver and intestine per mouse in infected untreated group was 54.801. It was decreased (2925 and 1900 respectively) in those treated intrapretonially and orally with aqueous extract of *R.nilotica*, and it was 41.074 per mouse in those treated intrapretonially with methanol extract of *R.nilotica*. It was 47.112 per mouse in those treated with praziquantel. (Fig2).

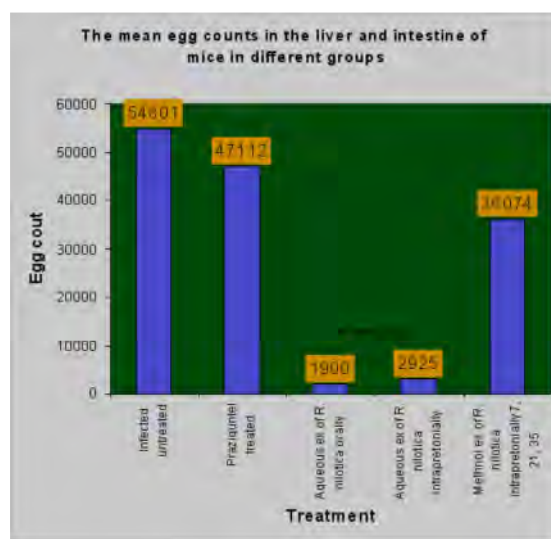


Fig 2: The mean egg content in liver and intestine.

The eggs collected from orally treated mice with aqueous extract were elongated and 5 trials were done for hatching by putting those eggs in warm water (40 C) under direct light for 2-3 hrs and no hatch occurred at all.

Discussion:

It is clear from the above results that the extracts of the *R. nilotica* showed significant effects on migrating juvenile of *S. mansoni* as it reduces the total and female number of recovered worms. The early attack of migrating juveniles with aqueous extract of *R. nilotica* resulted in significant effect by reducing the size of worms and decreasing the number of the egg counts in the liver and intestines. No hatch of eggs indicates clearly the strong effects (the reducing effect on the oogenesis process and this may be attributed to the effects of the extract on egg shells) of the *R. nilotica* on migrating juvenile and this may be the critical point which can cut off the life cycle of *schistosoma*. In the future, research should elucidate this point. Clinical signs and post mortem lesions which were not noticed in treated animals by all regimen of administration of extract supports the effects of the extract on pathogenesis of the worm and that also means the extract decreases the virulence of the worms. It is known that Praziquantel has no effects on immature worm²⁻³; the same results were obtained in this study under Sudan epidemiological condition.

R. nilotica is a highly promising plant in early therapy of Schistosomiasis.

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