Efficacy of *Randia nilotica* methanol extract against *Schistosoma mansoni* infection in mice

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Abstract:

Introduction:

Schistosomiasis is an important parasitic disease in the tropics. Emergence of praziquantel-resistence strains urged the need for new drugs.

Objective

To scientifically evaluate the effectiveness of a plant *(Randia nilotica*) used traditionally to treat schistosomiasis

Methods

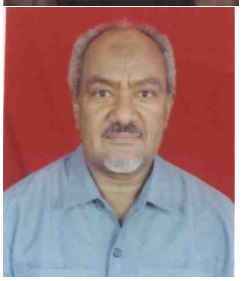
Albino mice were experimentally infected with single dose of 150 cercariae of the Sudan strain of Schistosom mansoni. All the cercariae penetrated the shaved tail of the mouse. The mice were treated with single i.p (intraperitoneal) dose of 1 ml of *R. nilotica* methanol extract (prepared from fruit part of the plant) in concentration of 5000 ppm and double doses through the same route of administration with 1 ml of R. nilotica methanol extract in concentrations of 1000 and 500 ppm.

Results

This resulted in total worm burden reductions at 87% 76% 68% respectively. The reductions in female worm burden were 99%, 97%, and 95% respectively. Oral administration with the same concentrations (single dose of 5000 ppm and double doses of 1000 and 500 ppm) resulted in total and female worm burden reductions. There was obvious reduction in the number of eggs in liver and intestinal tissues of the treated mice and improvement of their health when compared with the control group

Conclusion

We conclud that the methanol extract of R. nilotica is effective against S. mansoni



Introduction:

Schistosomiasis is an important parasitic disease in the tropics with huge impact on the socio-economic development of affected regions and its control is mainly with chemotherapy. Praziquantel is being used as the drug of choice[1]. Indications of resistance from field trials and laboratory studies had been demonstrated in mice[2-8]. Although these observations are not of any clinical significance so far, yet they indicate the importance of closely monitoring the efficacy of Praziquantel in different epidemiological settings, and stress further need for research and development of novel antischistosomal drugs[6, 9-11]. Doenhoff, et al[12], stressed the need for alternative drugs to treat Praziquantel–resistant schistosomes, such as already existing in Northern Senegal. *R. nilotica Stapf. bark* are used in treatment of jaundice in Jebal EL Nuba area (Western Sudan).

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It is collected by Bashir[13] for phytochemical screening which showed that its active principles are triterpeniod saponins. It gave alkaloid responses and its bark afforded ranges of hydrocarbons, sterols, simple triterpenes (both free and esterified) and fatty acids. Mannitol, scopoletin, scopolin, umbelliferone, iso-scopoletin and syringic acid were isolated from the methanol extract of the bark which was found to be rich in triterpene acids of the oleanane and ursane series.

Suliman et al[14], investigated the effects of R.nilotica on egg-hatching cercariae and miracida of S. mansoni. They found that it has effect against both stages at a high concentration of 10,000 ppm. El-Kheir and El-Tohami[15], reported that *Randia nilotica* is highly potent molluscicides. Elsheikh [16] screened forty plants used in Sudanese folk–medicine for the control and chemotherapy of schistosomiasis and recommended further investigations of the crude extract from fruit part of R. nilotica as chemotherapeutic agent for schistosomiasis. In this study, we investigated the effect of R. nilotica methanol extract on S. mansoni in infected mice given by the oral or intrapretonial routes. The criteria of investigation included reduction in total and (female) worm burdens, counting of eggs in tissue of liver and intestine and post mortem lesions.

Materials and methods:

Plant material:

Fruits of *Randia nilotica* were collected from Western part of Sudan (Kordofan Province) and Eastern Sudan (Angesna Mountains). The collection was carried out by the staff of the Medicinal and Aromatic Plants Research Institute (MAPRI).The methanol extract was prepared by mixing 350 grams of coarsely powdered fruit part of the *R nilotica* with 99% methanol for 24 hr using a soxhlet apparatus. The extracted substance was filtered and put in rotating water evaborator for solvent evaboration over-night and the extract was kept at 4^{o}C for biological studies. To prepare the stock solution 1gm from the extract was dissolved in 100 ml of distilled water and further serial dilutions were done using the stock.

Animals and experimental design:

Seventy male and female albino mice, weighing 20-25 gm, were used in this study. Animals were divided into seven equal groups; each mouse was infected with 150 S. mansoni cercariae for 1 hour by tail immersion method[17]. On day 42 after infection, three groups of mice were treated with single i.p dose of 1 ml of R. nilotica methanol extract in concentration of 5000 ppm and double doses by the same route of administration with 1 ml of *R. nilotica* methanol extract in concentrations of 1000 and 500 ppm each concentration for each group. On the same day after infection, three groups of mice were orally administrated with the same concentrations (single dose of 5000 ppm and double doses of 1000 and 500 ppm) for each group. Mice, in 7th group were infected but not treated and kept as control.

Parasitological techniques:

Schistosoma mansoni cercariae were obtained by infecting Biomphalaria pefeffri snails collected from Elkryab village (North of Khartoum) with miracidia obtained from the faeces of school children infected with S. mansoni at Elsiraha village, Gezira Province, Sudan. Fecal eggs were detected by the standard method[18] and tissue egg counts by the KOH digestion method previously described by[19]. Worms were recovered and counted by the perfusion techniques described before[20]. Control group was sacrificed, observing the standard animal rights legisilations, perfused and all the worms were collected and counted after 56 days from infection. All treated groups were also sacrificed, perfused and all the worms were collected, differentiated into males and females and counted after one month from the final extract administratoin.

Postmortem lesions:

Where mice were sacrificed for worm retreval and all observed lesions were recorded.

Statstical analysis:

The effect of treatment with *R. nilotica* was assessed by comparing the mean number of total worms in all of treated groups with the control group. For statistical analysis, student's t-test was used.

Results:

Single i.p dose of 1 ml of plant methanol extract at 5000 ppm 42 days after infection resulted in total worm burden reduction of 87% and female worm burden reduction of 99%. Three mice died after treatment. On the other hand, the oral administration of single dose of 1 ml of the same concentration of the extract at 42 days after infection resulted in total worm burden reduction of 85% and female worm burden reduction of 98%. Only 1 mouse died after treatment, (day 42 is chosen because it is the time of maturity of worms and onset of the disease which was confirmed by detection of eggs in faeces). The i.p administration of 1 ml of the plant extract in concentration of 1000 and 500 ppm for two successive days 42,43 after infection produced total worm burden reduction of 76% and 68% and female worm burden reduction of 97% and 95%, respectively. Two mice died after treatment with 1 ml of the plant extract in concentration of 1000 ppm. The oral administration of 1 ml of the plant extract in concentration of 1000 and 500 ppm for 2 days 42, 43 after infection resulted in total worm burden reduction of 70% and 61% and female worm burden reduction of 96% and 94%, respectively. No death among the mice occurred in the latter group. The mice with severe infection which resulted in large abdomen, pale colour, rough coat and rectal prolapse died. This could be attributed to their low immunity towards infection rather than to treatment, because the experiments done for toxicity of the extract in uninfected mice with the same dosage did not cause death[21]. The mean egg counts in liver and intestine per mouse in infected untreated control group was 54801 while it was only 3,971 and 6,127 eggs in liver and intestine of mice treated i. p and oral with 500 ppm methanol extract respectively which was significant decrease ($P < 0.05$). In the treated

Table 1. S. mansoni infected mice treated intrapretoneally with different concentrations of *R. nilotica* methanol extract.

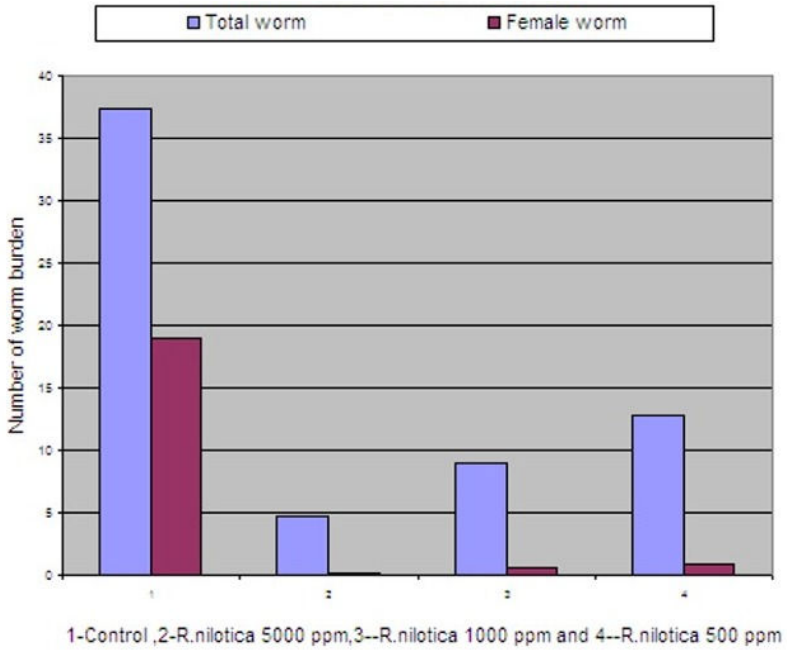
Group	Conc ppm	Administration after infection (days)	No. of mice	No. of mice died	Total worm burden Mean ±SD	Total worm burden reduction (%)	Female worm burden Mean ±SD	Female worm burden reduction (%)
1	Control	_	10	_	37.4± 7.7	_	19± 5	_
2	5000	42	10	3	4.7± 0.5*	87	0.2± 0.4*	99
3	1000	42,43	10	2	9± 5*	76	0.6± 0.5*	97
4	500	42,43	10	_	12.8± 8.7*	68	0.9± 0.8*	95

* highly significant P<0.005.

Table 2. S. mansoni infected mice treated orally with different concentrations of*R. nilotica* methanol extract.

Group	**Conc** ppm	**Adminstration day after infection**	**Mice (n)**	**Mice (d)**	**total worm burden Mean ±SD**	**Total worm burden reduction (%)**	**female worm burden Mean ±SD**	**Female worm burden reduction (%)**
1	**control**	_	**10**	_	**37.4±7.7**	_	**19± 5**	_
2	**5000**	**42**	**10**	**1**	**5.7±2.9***	**85**	**0.4±0.5 ***	**98**
3	**1000**	**42,43**	**10**	_	**11.1±5.3***	**70**	**0.7±0.5 ***	**96**
4	**500**	**42,43**	**10**	_	**14.5±5..9***	**61**	**1.1±0.7 ***	**94**

* highly significant P<0.005



The figure shows S.mansoni worm burden in mice treated intrapretoneally with different concentrations of *R. nilotica* methanol extract.

group most of the worms recovered had stunted growth, appeared smaller in size, morphologically distorted and the mouth parts and the grooves were not clearly developed, they died within 1 hour while the worms recovered from the control group died after 4-5 hours in citrate solution. The health condition of the mice was improved after treatment. Table 1 and 2 and the figure summarize the results.

The postmortem lesions in the control group were typical lesions of S.mansoni as: enlarged mesenteric tissues, enlarged and inflamed haemmorrgic liver with pyogenic foci, enlarged spleen, congested mesenteric blood vessels and empty intestine. In the treated mice, mesenteric blood vessels and tissues were normal, liver was normal in size, colour and texture, normal spleen and intestine was filled with food.

Discussion

The plant kingdom remains a virtually untapped reservoir of new chemical compounds, some providing novel structures from which synthetic chemists may derive even more interesting compounds[14]. The results of our studies clearly showed that the toxicity of methanol extract of *R. nilotica* to the adult stage of S. manson is highly significant $P<0.005$ for all concentrations used in treatment of infection in mice. This was clear in the significant reduction of the number of worms recovered from mice treated via both routes oral and i.p. The worms recovered from mice treated via i.p were lower than from the mice treated orally. This may be attributed to low absorption of extract from the intestines and/or the action of gastric juice on the extract. However, more investigations on the absorption of the plant extract and the use of a larger number of animals are needed for further studies. We conclud that the methanol extract of *R. nilotica* is effective against S. mansoni. It could contribute to the current rational research of alternative drug for Praziquantel resistant strains. Also we advice further researsh using different regements.

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