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

Saada M. Nour¹, Mohamed Magzoub² and Elamin I. Elnema³

Abstract:

**Introduction:**
Schistosomiasis is an important parasitic disease in the tropics. Emergence of praziquantel-resistance 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 conclude 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 choice¹. Indications of resistance from field trials and laboratory studies had been demonstrated in mice²⁻⁴. 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 drugs⁶⁻⁹,­¹¹. Doenhoff, et al¹², stressed the need for alternative drugs to treat Praziquantel–resistant schistosomes, such as already existing in Northern Senegal. *R. nilotica* Staf. bark are used in treatment of jaundice in Jibal EL Nuba area (Western Sudan).

1. Assistant Professor of Microbiology, Dept. of Microbiology, Faculty of Medicine, University of Upper Nile, Sudan.
2. Professor of Parasitology, Dept. of Parasitology, Faculty of Veterinary medicine, University of Khartoum, Sudan.
3. Associate Professor of Microbiology Dept. of Microbiology, Faculty of Pharmacy, University of Khartoum, Sudan.

Correspondence address:
Saada M. Nour.
Yahiai@ yahoo.com .Mobile: 0912812780, or 0122547151. Home :0183487110

It is collected by Bashir¹³ for phytochemical screening which showed that its active principles are triterpenoid 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 al¹⁴, 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-Tohami¹⁵, 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 intraperitoneal 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 evaporator for solvent evaporation over-night and the extract was kept at 4°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. 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 administered 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 pfeifferi snails collected from Elkryab village (North of Khartoum) with miracidia obtained from the faeces of school children infected with *S. mansoni* at Elsraha village, Gezira Province, Sudan. Fecal eggs were detected by the standard method and tissue egg counts by the KOH digestion method previously described. Worms were recovered and counted by the perfusion techniques described before. Control group was sacrificed, observing the standard animal rights legislations, 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 retrieval and all observed lesions were recorded.

Statistical 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. 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 intraperitoneally 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 | Administration 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 intraperitoneally 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 hemmorrhagic 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. The results of our studies clearly showed that the toxicity of methanol extract of R. nilotica to the adult stage of S. mansoni 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 conclude 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 research using different regiments.

Acknowledgements:

We are grateful to the staff of the Medicinal and Aromatic Plant Research Institute, Khartoum, for providing R. nilotica and to the staff of the Department of Pathology, Faculty of Veterinary Medicine, University of Khartoum, for giving us permission to use their aquarium for maintenance of the snails. We acknowledge financial support from M.M. Pharma. We would like to thank Dr. Yahia Fadl Tahir (University of Khartoum, Dept. of Archaeology) for his assistance in laboratory techniques.

References:

1. Report of the World Health Organization. Informal Consultation on Schistosomiasis control. Geneva (1999), WHO/CDS/CPC/SIP/99.2.
2. Fallon PG. Doenhoff M J. Drug-resistant schistosomiasis: resistance to Praziquantel and Oxamnique induced in Schistosoma mansoni in mice is drug specific .Am J Trop Med Hyg 1994; 51: 83-88.
3. Ismail M. Botros S. Metwally A. et al. Resistance to Praziquantel: direct evidence from Schistosoma mansoni isolated from Egyptian villagers. Am J Trop Med Hyg 1999; 60: 932-935.
4. Ismail M. Metwally A. Farghaly A et al. Characterization of isolate of Schistosoma mansoni from Egyptian villagers that tolerate high doses of Praziquantel. Am J Trop Med Hyg 1996; 55: 214-218.
5. Ismail MM. Taha SA. Farghaly AM. Laboratory-induced resistance to Praziquantel in experimental schistosomiasis. J Egyptian Society of Phraseology.1994; 24: 685-695.
6. Geerts S. Gryseels B. Drug resistance in human helminthes: current situation and lessons from livestock. Clinical Microbiology Reviews. 2000; 13: 207-222
7. William S. Sabra A. Ramzy F. Stability and reproductive fitness of Schistosoma mansoni isolates with decreased sensitivity to Praziquantel. International Journal for Parasitology 2001; 31: 1093-1100.
8. Danso-Appiah A. De Vlas SJ. Interpreting low Praziquantel cure rates of Schistosoma mansoni infections in Senegal. Trends in Parasitology. 2002;18: 125-129.
9. Cioli D. Chemotherapy of schistosomiasis: an update. Parasitology Today 1998; 14: 418-422.
10. Cioli D. Praziquantel: is there real resistance and are there alternatives? Current Opinion in Infectious Diseases 2000; 13: 659-663.
11. Gryseels B. Mbaye A. De Vlas SJ. Are poor responses to Praziquantel for the treatment of Schistosoma mansoni infections in Senegal due to resistance? An overview of the evidence. Trop Med Internat Health 2001; 6: 864-873.
12. Doenhoff MJ. Kusel JR. Coles G.C. Resistance of S. mansoni to praziquantel: is there a problem? Trans R Soc Trop. Med. Hyg. 2002; 96: 465-9.
13. Bashir AK. Pharmacognostical studies on the Sudanese Medicinal plants Randia nilotica staph and Grewia villosa Wild1980; Ph.D. thesis University of Wales. U.K.
14. Sulaiman MS. Ahmed K. Bashir AK. Effects of certain Sudanese plant extracts on egg-hatching, miracidia and cercariae of S.mansoni. Int.J.Crud. Drug Res. 1988; 26:17-21
15. El-Kheir YM. and El-Tohami M S. Investigation of molluscicidal activity of certain Sudanese plants used in folk-medicine. J. Trop. Med. Hyg. 1979; 82: 237-247.

16. Elshiekh HS. Biological and Chemical Studies on Molluscicidal and Schistosomiasis Compounds. 1994. Ph.D. thesis University of Khartoum, Sudan.

17. Magzoub M Magraithe BG. Glycolytic activities of Schistosoma mansoni and the mode of action of antischistosomal drugs (Ambilhar and Astiban). Ann Trop Med Parasitol 1969; 63: 377-391.

18. Katz N. Chaven A. Pellegrino J. A simple device for quantative stool thick-smear technique in Schistosomiasis mansoni. Revista do Instituto de Medicina Tropical de Sao Paulo 1972; 14: 397-400.

19. Cheever AW. Conditions affecting the accuracy of potassium hydroxide digestion techniques for counting S. mansoni eggs in tissues. Bull. WHO. 1968; 39: 328-331.

20. Smithers SR. Terry RJ. The infection of laboratory hosts with cercariae of S. mansoni and the recovery of the adult worms. Parasitol. 1965; 55: 696-700.

21. Saada M N. Efficacy of Three Sudanese Medicinal plants against adult Schistosoma mansoni in comparison with Praziquantel, 2005. Ph.D. thesis, University of Khartoum, Sudan.