The In Vitro Effects of Graded Concentrations of Methanol Extract of Randia Nilotica Plant on the Utilization of Exogenous Glucose by Schistosoma Mansoni Worms.

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Abstract:
Introduction: The efficacy of Methanol extract of R. nilotica plant in treatment of mansoni schistosomiasis in comparison with praziquantel drug in mice has been evaluated.
Objective: To investigate how Randia nilotica eradicates schistosoma mansoni.
Materials and Methods: The extract of Randia nilotica was obtained by a routine measurement. Cercariae of S. mansoni were obtained by infecting Biomphalaria pfeifferi snails with miracidia. Fecal eggs were detected by the standard methods Mice were infected by 156 cercariae for each mouse using padding method. The worms were recovered from those infected mice from the mesenteric and portal veins by the perfusion techniques and were incubated in Bueding medium at 37°C. The same experiment was repeated after adding graded concentrations of the methanol extract to the medium. Quantitative analysis of glucose in the media was carried out.
Results: The mean percentage of glucose uptake by the normal worms from the medium after four hrs of incubation was 36% where as that of worms exposed to different concentrations (50 ppm, 500 ppm, 2000 ppm & 5000 ppm) of methanol extract of R. nilotica were 26%, 8%, 6%, 5% respectively.
Conclusion: The inhibition of glucose uptake by this extract may be a major factor in the eradication of the worms. However, reaching that conclusion mandates prior knowledge of the effects of the R. nilotica on the enzymes and substrates of the glycolitic process of S. mansoni. The possibility of purifying an extract as a new drug against S. mansoni from this plant has to be considered.

Introduction
Schistosomiasis is the second commonest parasitic diseases in the world after malaria, in terms of the extent of endemic areas and the number of people infected. In view of the possible tolerance or resistance to praziquantel, the drug of choice in treatment of Schistosomiasis research to produce new drugs for the prevention and cure of praziquantel – resistant Schistosomes, such as already exists in Northern Senegal has become justified.

The efficacy of Methanol extract of R. nilotica plant in treatment of mansoni schistosomiasis in comparison with praziquantel drug in mice has been evaluated and resulted in 87% and 59% inhibition of worm-load respectively. Bueding and Oliver-Gonzales, believed that anaerobic metabolism, particularly glycolysis, rather than respiration, supplies the energy necessary for the survival of S. mansoni.

Objective
The metabolism of the parasitic helminthes, as a group, is of particular interest because of the differences in the chemical composition of the habitat of various species. Since Schistosoma is a blood fluke, it depends mainly on exogenous glucose up-take from the blood for obtaining energy.

The work presented in this paper deals with the study of the up-take of exogenous glucose by the normal worms and by those incubated with graded concentrations of Methanol Extract of Randia nilotica.

Materials and Methods
This research was done in the Department of Microbiology, Faculty of Pharmacy, University of Khartoum, Sudan.

The plant
Fruits of Randia nilotica were collected from Eastern Sudan (Angesna Mountains). The collection was carried out by the staff of the Medicinal and Aromatic Plants Research Institute (MAPRI). The plant extract was obtained by a routine measurement.

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Schistosoma mansoni worms

Cercariae of S. mansoni were obtained by infecting Biomphalaria pfeifferi snails collected from Elkriyab (Khartoum) with miracidia obtained from the feces of school children infected with S. mansoni at Elsiraha (Gezira, Sudan). Fecal eggs were detected by the standard method. Mice were infected by 150 cercariae for each mouse using paddling method. The worms were recovered from those infected mice from the mesenteric and portal veins by the perfusion techniques.

The animals

The animals used were white albino mice, of both sexes and of 20-25 gm. weight. These mice were bred and maintained on the regular laboratory diet, in the Faculty of Pharmacy of Khartoum University.

Incubation Medium and Washing Medium

The medium used for the incubation of the worms was Bueding medium. It contains 100 mg glucose. The citrate saline was used for washing the worms.

Preparation of Worms for Incubation

After collection and washing in citrate saline, the worms were transferred by a Pasteur pipette, with the minimum possible citrate saline, to Bueding medium already incubated at (37°C for 80 minutes). The worms were counted in pairs and placed in 15 carrel flasks, each flask received 10-15 pairs of worms. The flasks were incubated for four hours. The glucose uptake was measured in all flasks at the end of incubation.

The plant extract was prepared by dissolving it in Bueding medium. The concentrations of the extract (5000 ppm, 2000 ppm, 1000 ppm, 500 ppm and 50 ppm) were transferred into each flask to which 100 mg of glucose were added. The flasks were numbered, plugged, surrounded by cotton wool and put in the incubator at 37°C for four hours.

Determination of Glucose

Quantitative analysis of glucose in the medium was carried by means of a sensitive enzymatic method (Tc-M (Boehriger & Soehne GMBH, Manheim Germany).

Plan of the Experiments

Experiment 1

Was designed to study: The glycolysis of S. mansoni worms in terms of glucose uptake from the media containing glucose by worms, picked from infected (after 10 weeks of infection) but not treated mice, after 4 hrs of incubation at 37°C in glucose containing media.

Experiment 2

Was designed to study the effects of graded concentrations of the methanol extracts of Randia nilotica on glucose uptake by S. mansoni worms after four hours of incubation at 37°C in glucose containing medium.

Results

The results of the media analysis for glucose uptake at the end of incubation (4hrs) showed that the worms took up glucose actively from the medium.

The mean of glucose uptake by an average of 12 pairs of worms after four hrs of incubation were 1434.8 ugm.

The rest of the glucose which remained in the solution in the flask indicated that, excess glucose in the environment did not induce a higher uptake by the worms.

The mean uptake of glucose by a single pair of worms in four hrs was 115.4 ugm.

The mean percentage of glucose uptake by the normal worms from the medium after four hrs of incubation was 36%.

The mean percentages of glucose uptake by the worms exposed to different concentrations (50 ppm, 500 ppm, 2000 ppm & 5000 ppm) of methanol extract of R. nilotica were 26%, 8%, 6%, 5% respectively (Graph 1).

![Graph](image)

Fig. Glucose uptake by adult S. mansoni exposed to different concentration of R. nilotica extract in media containing glucose.

All results are summarized in Table (1).
Table 1: Effects of graded concentration of methanol extract of *R. nilotica* on glucose uptake by *S. mansoni* worms in different experiments.

<table>
<thead>
<tr>
<th>Group/ conc.</th>
<th>No of Experiments</th>
<th>No of pairs</th>
<th>Glucose uptake g/mg/flask</th>
<th>Glucose uptake g/mg/pair</th>
<th>Average glucose uptake %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control(00) ppm</td>
<td>15</td>
<td>174</td>
<td>1434.8</td>
<td>115.42</td>
<td>35.93%</td>
</tr>
<tr>
<td>G1 (50) ppm</td>
<td>10</td>
<td>101</td>
<td>951</td>
<td>100.13</td>
<td>25.7%</td>
</tr>
<tr>
<td>G2 (500) ppm</td>
<td>10</td>
<td>76</td>
<td>312.00</td>
<td>51.30</td>
<td>7.8%</td>
</tr>
<tr>
<td>G3 (2000) ppm</td>
<td>5</td>
<td>50</td>
<td>248.0</td>
<td>24.50</td>
<td>6.4%</td>
</tr>
<tr>
<td>G4 (5000) ppm</td>
<td>10</td>
<td>100</td>
<td>222</td>
<td>22.1</td>
<td>5.4%</td>
</tr>
</tbody>
</table>

**Discussion**

Magzoub and Maegraith\(^{10}\) presented some useful information on the effects of Niridazole and Stibocapate (drug of choice in treatment of Schistosomiasis at that time) on the exogenous glucose uptake by *S. mansoni* adult worms. Graded concentrations of both drugs were used. Niridazole at 2 mg%, 6 mg% and 10 mg% had inhibited glucose uptake by 64.4%, 67.4% & 67.6% respectively. Further studies on the effects of these drugs on other products of the glycolytic process of the worms proved that the inhibition of glucose uptake by both drugs was mainly due to the inhibition of the enzymes hexokinase and phosphofructokinase respectively\(^{10,11}\).

In the present work, the effects of the methanol extract of *Randia nilotica* on *S. mansoni* adult worms were studied. The same in vitro conditions employed by the above mentioned authors were applied in the present work. The inhibition of glucose uptake by *S. mansoni* under the influence of the methanol extract of *Randia nilotica* (using 50 ppm, 100 ppm, 2000 ppm & 5000 ppm) was found to be higher than the inhibition of the up-take by *S. mansoni* under the influence of the Niridazole and Stibocapate using different concentrations. It seemed that the drugs (Niridazole and Stibocapate) and the plant *R. nilotica* had shown increasing rates of inhibition of glucose uptake by the parasite following an increase in their concentrations.

**Conclusion**

It was clear from the results of glucose uptake inhibition that the incubation in lowest concentration of 50 ppm of *R. nilotica* extract resulted in 34% inhibition and it increased with the increment of the extract concentration to 78%, 83% and 85% respectively. The inhibition of glucose uptake by this extract may be a major factor in the eradication of the worms. However, reaching that conclusion mandates prior knowledge of the effects of the *R. nilotica* on the enzymes and substrates of the glycolytic process of *S. mansoni*. The possibility of purifying an extract as a new drug against *S. mansoni* from this plant has to be considered.

**References**