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Chemotherapeutic Effect of Niridazole (Ambilhar–Biomedean Belgium) on Schistosoma Mansoni Infection in Mice.

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ABSTRACT

The chemotherapeutic effect of niridazole on Schistosoma mansoni in mice was evaluated at 2 weeks, 4 weeks and 8 weeks post cercarial exposure. The schistosomicidal effect of niridazole increased with increasing doses of the drug at 2 weeks and also at 8 weeks post cercarial exposure ($p<0.05$). Worm fecundity was more affected when the drug was given to the 2 weeks old worms than the 4 weeks and the 8 week–old worms although, oogram patterns of the 8 week–old worms revealed that the eggs in the tissues were killed in that about 89.41% dead eggs were found and there was absence of 2nd and 3rd stage eggs in the intestinal segments.

A dose of 5.0mg/ml given orally daily for 5 days can effectively control S. mansoni infection in the 2 weeks and 8 weeks old infections.

INTRODUCTION

Chemotherapy and chemoprophylaxis are known to play the most important and crucial role in the control of schistosomiasis, a grave and debilitaing disease of socio–economic importance[1]. Most of the synthetic orally administered and well tolerated schistosomicides in use include, praziquantel[2], Oxaminiquine[3] Amoscanate[4] and Niridazole[5]. Niridazole was shown to be active against *S. mansoni, S. hematobium* and *S. japonium*, especially the female worm[6,7]. Different geographical strains of *S. mansoni* were reported to respond variosly to niridazole [8,9]. Taylor and Nelson[8] reported that *S. mansoni* strains from Liberia were more sensitive to the drug than strains from Tanzania.

Although Praziquantel is reported to be the most effective drug for *S. mansoni* and other trematodes, its use is limited by the cost of treatment. Niridazole could thus be a cheaper alternative treatment for bilharziasis and therefore the need to further experiment on the effect of the drug on the different geographical strains of the parasite. This study thus aims at studying the chemotherapeutic effect of niridazole on the different developmental stages of *S. mansoni* in mice, including the reproductive potential of treated parasites.

MATERIALS AND METHODS

Animals

Adult white mice weighing between 19.5g and 23.5g and the Nigerian strain of *Schistosoma mansoni* were used.

Naturally (field) infected *Biomphalaria pfeifferi* were collected from Plateau State of Nigeria. The snails were induced to shed cercariae by exposing the snails to light from a 60 – watt electric buld. The mice were infected with the cercariae, using the paddling method of Moore et al[10]. Each mouse was exposed to between 120 – 150 cercariae.

Drug Preparation

Niridazole (Ambilhar – Biomedieane Belgium) 500mg tablet was crushed into fine powder and suspended in 100ml of distilled water. This suspension was further diluted to obtain the following concentrations of the drug: 5.0mg/ml,; 3.0mg/ml; 2.5mg/ml and 1.5mg/ml. All suspensions were kept in the refrigerator until required, for up to 14 days after which it was discarded and a fresh preparation was made. The drug was orally administered by placing the appropriate dose directly into the stomach using a blunt needle and syringe.

Experimental Procedure

Twelve groups of infected mice, each consisting of 8 mice at different developmental stages, namely at 2, 4 and 8 weeks were individually administered the following concentrations of niridazole, orally once daily for 5 days; 5.0mg/ml; 3.0mg/ml, 2.5mg/ml, 1.5mg/ml. This is referred to as one course of treatment. Parallel infected, but untreated mice were set aside as controls. Eight weeks after cercarial exposure, 5 of the mice dosed at 2 weeks and at 4 weeks of age were sacrificed by cervical dislocation. For the 8 week–old worms, mice were sacrificed 7 days after administration of the drug. Control mice were also sacrificed when worms matured at 8 weeks.

The organs of the mice, namely the intestine, liver and lung were carefully removed and placed in separate petri–dishes containing physiological saline. The organs were meticulously teased out and the worms were searched for and counted. The effect of the drug was evaluated in relation to the control worms.

Determination of the Effect of Niridazole on the egg and fecundity of S. mansoni

The average egg load in the liver of the treated mice was determined by the tissue digestion in potassium hydroxide technique described by Cheevers[11]. The different morphological egg froms laid in the intestinal tissue by S mansoni following treatment was studied using the oogram technique for egg classification described by Pellegrino et al[12]. Six distinct morphological forms of eggs were found. The percentage occurrence of each was determined. This constitutes the Oogram profile for each drug concentration that was administered. That of the control was also determined.

RESULTS

Effect of age of S. mansoni on the schistosomicidal action of niridazole in mice.

The results presented in table I show that the average worm recoveries from mice harbouring different ages of S. mansoni decreased as the drug concentrations increased, except for the 4 week–old worms where administration of a course of 3.0mg/ml resulted in the recovery of 70.55% as compared with the 63.38% recovery from mice administered 2.5mg/ml. The schistosomicidal effect was greater in the 2 week–old worms than the 4 week–old parasites and it increased in the 8 weeks–old parssites. While 2 week–old worms show a statistically significant dose – worm recovery relationship ($p<0.05$), the 4 week – old worms showed less susceptibility to niridazole ($p > 0.05$).

One course of 5.0mg/ml of niridazole completely cured the mice with the 8 week–old parasites, while it caused a 7.42% recovery in the 2 week–old and 45.72% in the 4 week–old parasites.

Effect of niridazole on the fecundity of S. manson in mice

Table II shows the effect of niridazole on the total egg load of parasites after treatment at 2 weeks, 4 weeks and 8 weeks post cercarial exposure. Fecundity was greatly affected for the 2 week–old parasites by all he concentration of the drug used.

The course of 1.5mg/ml caused 93.82% reduction in fecundity while 5.0mg/ml caused a 100% reduction and the dose fecundity relationship was statistically significant ($p<0.05$).

For the 4 week–old worms a course of 1.5mg/ml did not appear to have had any effect on worms fecundity but 5.0mg/ml caused a 60.72% reduction in the liver egg load. Again the effect of the drug on fecundity is not statistically significant ($p>0.05$) for the 8 week–old worms, although the liver egg load was reduced, it could not be accounted for, by the effect of the drug since mice were sacrificed only a few days after dosing.

Effect of Niridazole on S. mansoni eggs in the intestinal wall.

Table III shows the quantitative counts of the different egg forms of S. mansoni in relation to the different doses of niridazole administered. For the mice dosed with one course of 1.5mg/ml niridazole at 2 weeks of infection there was absence of the second stage developmental forms. A course of 3.0g/ml at this stage revealed absence of first and 4th stage egg forms in the intestinal wall. That is, there was a cessation of egg production. This is indicative that the drug did affect the reproductive organs of the female worms at 2 weeks post cercarial infection. The oogram profile of 4 weeks worms in mice revealed that only a course of 5.0mg/ml caused the absence of first and second stage eggs and the lower concentrations did not alter the oogram profile. At 8 weeks of age, drug administration even at lower doses resulted in very high percentage of dead eggs, thus, a course of 1.5mg/ml resulted in 89.41% dead eggs, for all the eggs counted in the tissue. All the doses of drug administered to the 8 week–old worms caused an alteration in the oogram profile. The reason for this is discussed. The oogram profile for the 2 week, 4 week and 8 week – old worms in intestinal tissues for all the concentrations studied is presented in the Figs I–III. The curves revealed that the occurrence of the different developmental forms is not affected by the dosage of the drug for the 2 week–old worms. The same can be said for the 4 week and 8 week–old worms.

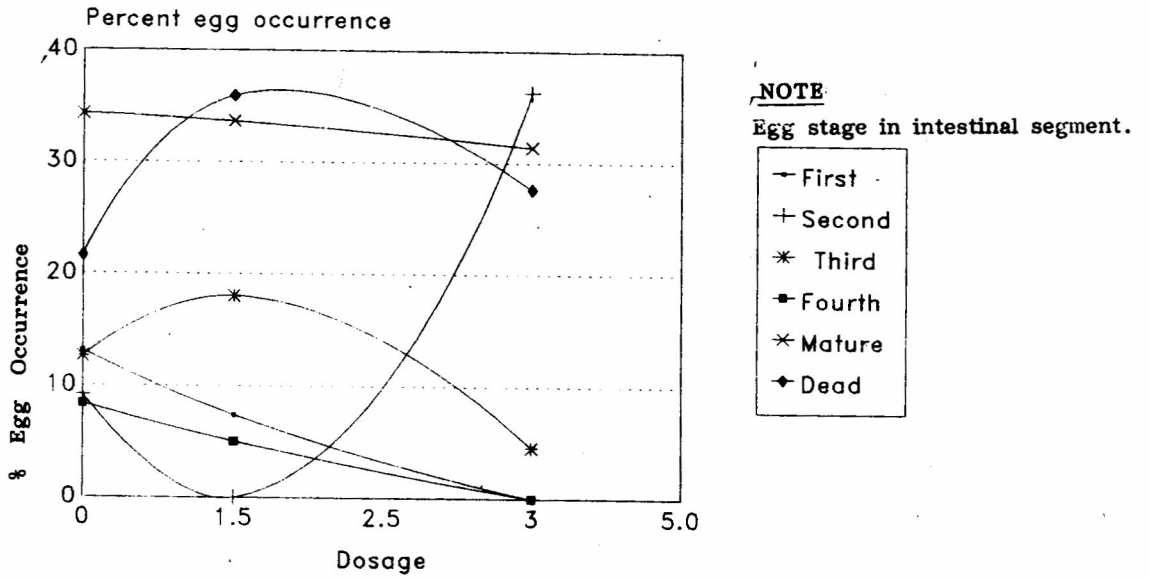

Fig. 1: The effect of ambilhar on the oogram pattern of *S. mansoni* infections in mice at 2 weeks of age

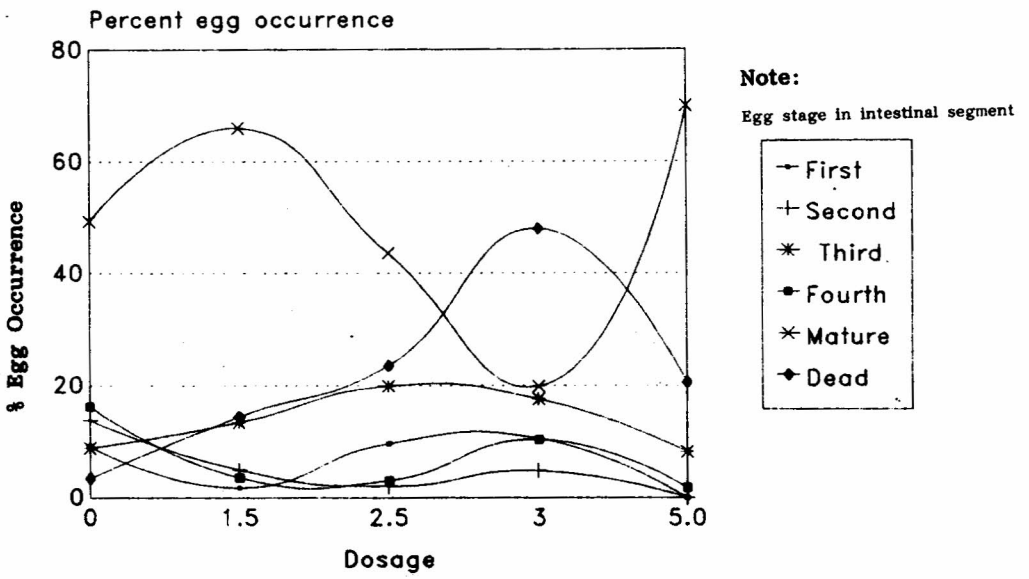

Fig. 2: The effect of ambilhar on the oogram pattern of *S. mansoni* infections in mice at 4 weeks of age.

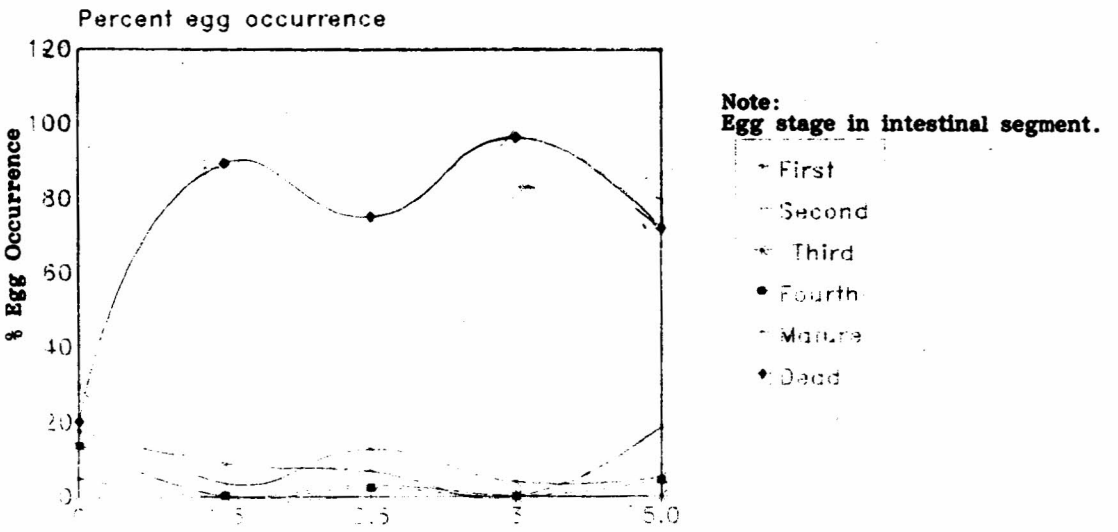

Fig. 3: The effect of ambilhar on the oogram pattern of *S. mansoni* infections in mice at 8 weeks of age.

Table I

The Schistosomicidal effect of Niridazole on S. mansoni at 2 weeks, 4 weeks and 8 weeks post cercarial expoure.

Concentration of Niridazole mg/ml for 5 days	No. of Mice use	TIME OF TREATMENT AFTER CERCARIAL – EXPOSURE					
		2 Weeks		4 Weeks		8 Weeks	
		Average worm Count per mouse	% Recovery	Ave. worm count per mouse	% Recovery	Ave. worm count per mouse	% Recovery
CONTROL	10	89.5	100	38	100	171.5	100
1.5	10	24.2	27.04	26.75	69.48	91.1	52.99
2.5	10	18	20.67	24.4	63.38	77.5	45.19
3.0	10	15	16.76	27.16	70.55	72	41.98
5.0	8	6.6	7.42	17.6	45.72	0	0.000
		($p<0.05$)		($p>0.05$)		($p<0.05$)	

Table II

The Average liver egg load of S. mansoni in Mice Treated after 2 weeks, 4 weeks and 8 weeks post infection

Concentration of Niridazole mg/ml x 5 days	Total Mice used	AVERAGE LIVER EGG LOAD PER MOUSE								
		2 Weeks			4 Weeks			8 Weeks		
		Treated	Control	% Difference	Treated	Control	% Difference	Treated	Control	% Difference
1.5	2	742.4	12,012	93.82	5436	4392	0	2549	5776	55.86
2.5	2	511	6507	92.15	1756.8	3600	51.2	4229.4	14,546.4	70.92
3.0	2	840	21,284	96.05	3408.8	6120	44.33	5608.8	17,578.8	68.09
5.0	2	0	5124	100	1584	4032	60.71	3127.4	3127.4	69.29

Table III

Effects of various doses of ambilhar on the developmental stages of S. mansoni eggs in mice.

III A: Treatment given 2 weeks after exposure

Drug Dosage	Average % of egg developmental stages in intestinal segments					
mg/ml x 5 days	1°	2°	3°	4°	Mature	Dead
1.5	7.39	0	18.05	5.04	33.68	35.96
2.5	ND	ND	ND	ND	ND	ND
3.0	0	36.36	0	4.57	31.42	27.65
5.0	ND	ND	ND	ND	ND	ND
Control	13.12	9.18	8.39	12.61	34.33	21.63

III B: Treatment given 4 weeks after exposure

1.5	1.74	4.93	13.40	3.56	66	14.9
2.5	9.61	1.99	19.86	3.04	45.58	23.49
3.0	10.53	4.80	17.57	10.33	19.84	47.94
5.0	0	0	8.15	1.77	70.01	20.56
Control	9.13	13.76	8.80	16.21	49.3	3.43

III C: Treatment given 8 weeks after exposure

1.5	9.04	0	0	0.37	3.68	89.41
2.5	6.85	0	3	2.35	12.8	75
3.0	0	0	0	0	3.85	96.15
5.0	18.72	0	0	4.38	5.07	71.84
Control	17.74	14.46	5.08	13.8	29.58	20.19

ND = Not Done.

DISCUSSION

Niridazole in this study shows a schistosomicidal characteristic when higher doses of the drug was used and no residual dead worms were recovered. The worms recovered and counted were alive.

The effect of niridazole increased with increasing doses of the drug and the effect was more pronounced when administered to 2 week–old infections and the 8 week–old infections. However, when the drug was administered to 4 week–old infections, only higher doses of the drug significantly affected the worms.

Sabah et. al.[13] had reported that niridazole (200.mg/kg x 5) was effective against S. mansoni in the first and second weeks after infection and also in 6 week old infection but not on 3 week–old worms. Praziquantel was reported to have acted similarly to niridazole.

While there is inadequate explanation for the action of niridazole on the early infections, several reasons have been put forward for its action on the mature infections. It was reported that niridazole damages the tegument of the worms thus making them vulnerable to immune destruction[14]. It has also been shown that antibodies against S. mansoni adult worms are synergistically involed with drugs in the death of the worms[15].

The detoxification and metabolism of drugs occur in the liver and it has been reported that this function of the liver could be affected by concomitant disease in the organ or due to the embolization and granulomatous inflammation induced by the *S. mansoni* eggs[16,17]. This follows that the increased susceptibility of adult *S. mansoni* to niridazole may be due to the reduced capacity of the liver to convert the drugs to the inactive metabolites.

The action of niridazole on S. mansoni fecundity as shown in this study resulted in the reduction of the total liver egg load on the 2 week and 4 week–old worms. This is explained by the fact that the female S. mansoni reproductive system is shown to be highly susceptible to drug action[18,19]. It was demonstrated variously that the vitelline glands of these organs are mostly affected.

The quantitative study of the different morphological forms of *S. mansoni* eggs using the oogram technique further reveals the effect of niridazole on the worms at different stages of development. The oogram patterns for the various concentrations of the drugs used also revealed the susceptibility of *S. mansoni* female organs at the 2 week state as well as the 8 week (mature) even to low concentrations of the drug. However the 4 week–old worms were resistant to niridazole except the very high concentrations. Also, there was a very high value of dead eggs

in the oogram pattern of the 8 week–old worms, and it has been shown that niridazole kills the S. mansoni eggs within the tissues of the host[6,7].

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REFERENCES

1. Shekhar KC. Schistosomiasis drugs therapy and treatment considerations. Drug (1991); 42:379–405.
2. Andrew P., Thomas H., Pohlke R, Seubert., J. Praziquantel. Med Res (1983); 3:147–200.
3. Richards HC, Forster R. A new series of 3–aminomethyltetra–hydroquinoline derivatives displaying schistosomicidal activity in rodents and Primates Nature (London) (1969); 222:581–2.
4. Striebel H. P 4-1 sothio-cyanate-4nitrophenylamine (C9333–GCP 4540), an anthelminthic with an unusual spectrum of activity against nematodes, Filariae and Schistosomes, Experientia, (1976); 32:457–458.
5. Lambert CR. Chemotherapy of experimental S. mansoni infections with a nitrothiazole derivative. CIBA 32, 644-Ba. Ann. Trop. Med. Parasite (1964); 58:292–303.
6. Hess R, Faigle JW and Lambert C. Selective uptake of an antibiharzial nitrothiazole compound by S. mansoni. Nature (London) (1966); 210:964-965.
7. Monterio W, Pellegrino J. and Silva MLH. Unsual oogram pattern in mice after niridazole treatment. J. Parasit (1968); 54:175–176.
8. Taylor MG and Nelson GS. A comparison of the susceptibility to niridazole of two geographical strains of *Schistosoma mansoni* in *mice* with a note of the susceptibility of *S. Matheei.* Trans Roy. Soc. Trop. Med. Hyg. (1971); 63:169–174.
9. Bruce JI and EH Sadun. Prophylactic and Curative activities of antimony (3) dinner capto succinic acid in olive oil suspension, dicarbonic acid hydrazide derivative in rhesus monkeys experimentally unifected with *S. mansoni.* Am. J. Trop. Med. Hyg. (1966); 15:324–332.
10. Moore DV, Yolles TK. and Melene HE. A comparison of common laboratory animals as experimental hosts for *Schistosoma mansoni* J. Parasite (1949); 35: 156–170.
11. Cheever AW Relative resistance of the eggs of human schistosomes to digestion in Potassium hydroxide. Bull WHO (1970); 45:601–603.
12. Pellegrino J., Oliveire CA, Faria J and Cunha, AS. New approach to the screening of drugs in experimental Schistosoma mansoni in mice. Am. J. Trop. Med. Hyg. (1962); 11:201–215.
13. Sabah AA., Cathy Fletcher G. Webbe MJ. Doenhoff, *Schistosoma mansoni* Chemotherapy of infections of different ages Exptl Parasit. (1986); 60:348–354.
14. Huang TV Search for antischistosomal drugs on the basis of biochemicatry of schistosomes Arch. Pharm Chem. Sci. (1981); 9:1–24.
15. Doenoff MJ. (and Bain J. The immune dependence of schistosomicidal chemotherapy. Relative lack of efficacy of an antimonial in *Schistosoma mansoni* infected mice deprive of their of their T–cells and the demonstration of drugs antiserum synergy Clin. Exptl. Immunol. (1978); 33:3232–238.
16. Sherlock S. Factors determining the hepatic reactions to drugs. Ann. N.Y. Acad. Sci. (1969); 10:1775–782.
17. Cha YN and Edwards R. Effects of S.mansoni infections on the hepatic drug metabolising capacity of mice. J. Pharm. Exptl. Ther. (1976); 199:432–440.
18. Popiel I, and Erasmus DA. *Schistosoma mansoni:* Niridazole - induced damage to vitelline gland. Expt. Parasitol. (1981); 2:35.48.
19. Erasmus DA and Popiel I. Schistosoma mansoni: drug induced changes in the cell population of the vitelline gland. Expt. Parasit (1980); 50:171–187.
20. Levy LF. Bilharzia involvement of the central nervous system. Med. J. Zambia (1970); 14:191–99.