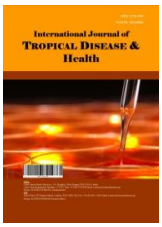

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Efficacy of Oral Artemisinin and Praziquantel Chemotherapy on *Schistosoma mansoni* Infected Mice by Means of Parasitological Parameters

Nassar, Adebayo Sulaiman[1], Bakarey, Adeleye Solomon[2*] and Abdulazeez, Abubakar Aderemi[3]

[1]Department of Medical Laboratory Sciences, Ladoke Akintola University of Technology, Ogbomoso, Oyo State, Nigeria.
[2]Institute for Advanced Medical Research and Training College of Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.
[3]Department of Medical Laboratory Science, University of Medical Science, Ondo, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. All authors designed the study, performed the statistical analysis, wrote the protocol and authors NAS and BAS wrote the first draft of the manuscript. Authors NAS and BAS managed the analyses of the study. Author AAA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Praziquantel-based chemotherapy is generally effective in the control of morbidity, decline in the prevalence and intensity of *Schistosoma mansoni* infections. Nevertheless, the potential emergence of praziquantel resistance in *S. mansoni* posses danger in the elimination of this neglected tropical disease in Africa. Therefore, this study was designed to evaluate the *in-vivo* efficacy of Praziquantel and Artemisinin using mice infected with *S. Mansoni* cercariae.
Methods: Infected mice with *S. mansoni* were treated with different doses of Artemisinin and Praziquantel from day 42 post infection to assess its efficacy on adult worms and eggs. A 2-day

**Corresponding author: Email: drbakarey@yahoo.com,drbakarey@gmail.com, asbakarey@com.ui.edu.ng;*

Protocol: artemisinin 400 mg/kg with Praziquantel of 500 mg/kg daily, 4-day protocol: artemisinin 200 mg/kg with praziquantel of 250 mg/kg daily, 6-day protocol: artemisinin 100 mg/kg with praziquantel of 125 mg/kg daily were used. A value of $p < 0.05$ was considered as the level of significance using statistical package for social sciences (SPSS) version 21.
Results: Of the 150 snails collected, 60(40%) were confirmed to be *Biomphalaria*, while 50(33.3%) and 40(26.3%) were *Bulinus* and *Intercalatum* respectively. Highest reduction of 66.3% was found in group II, compared with 58.8% and 56.5% significant reductions in groups I and III, respectively. Also, there were 77.8% and 74.2% significant reductions in eggs per gram of the small intestinal tissue noted in groups I and II respectively as against 63.1% reduction in group III. A significant decline in the percentage of total immature stages of 6.14% in group I was observed when compared to 66.14% in the control. Furthermore, a statistically significant boost of 57.57% was found in the protocol of Artemisinin 100 mg/kg with Praziquantel 125 mg/kg daily (6-day protocol) causing decreases of 43.9% and 42.4% in the total and female worm loads, respectively. The combination decreased intestinal tissue egg loads ranging from 63.1% to 77.8% and liver egg loads ranging from 56.5% to 66.3% rates.
Conclusion: Combined effect of the drugs has confirmed some level of efficacy on experimental *S. Mansoni* with significant reduction in tissue egg burden.

Keywords: Chemotherapy; artemisinin; praziquantel;Schistosoma mansoni; neglected illness.

1. INTRODUCTION

Schistosomiasis is a chronic and debilitating ailment caused by blood-dwelling trematode from the genus Schistosoma. The global health challenge of schistosomiasis is next to malaria according to World Health Organization [1]. An estimated 150,000 deaths per year in sub-Saharan Africa alone were attributable to schistosomiasis [2]. Also, people infected with schistosomiasis may have higher susceptibility to other infectious diseases such as HIV and AIDS [3]. This resulted up to 70 million disability-adjusted life years lost annually (DALYs). This figure exceeds some numbers of neglected tropical diseases [4]. Despite the high impact mentioned, the aggregate health problem of schistosomiasis is generally underestimated due to complexity of evaluating the disease [5].

Current treatment of schistosomiasis depends on praziquantel (PZQ) drug, produced in the late 1970s [6]. This drug has been generally used to control schistosomiasis. Conversely, PZQ is not indicated for the treatment of early infection or prevention of reinfection [7]. Additionally, available evidence shows the emergence of the drug resistance in *Schistosoma mansoni* [8]. For instance, investigations in Egypt, Senegal and Kenya revealed different degrees of PZQ drug resistance in *Schistosoma mansoni* [9]. A few antimalara drugs such as mefloquine and miltefosine have demonstrated anti-schistosomal activity [10]. Management of these drugs with or without PZQ is presently being evaluated [8].

Some new drug candidates such as anti-oxidant inhibitors namely oxadiazoles [11] and some protease inhibitors [12] have promising potentials against a schistosome infection, but have yet advanced to clinical trials. Owing to the emergence of resistant strains of Schistosomiasis to praziquantel which is the major drug for its treatment and the decreased effectiveness of other single drugs for managing this ailment, there is the need to study the effectiveness of the combined medications already in existence so as to boost drug efficiencies and to reduce resistance. This study therefore investigated the efficacies of Artemisinin and Praziquantel chemotherapy as anti-Schistosoma drugs in laboratory infected mice.

2. METHODOLOGY

2.1 Study Area

The study was conducted at the animal house of the Ladoke Akintola University of Technology, in Osogbo, Osun State, Southwestern Nigeria. Osogbo is the capital of Osun State found between longitude 4°34'E and latitude 7°46'N. The land mass of the town is about 47 km² with a population of 156,694. It is dominated by the Yorubas but with an admixture of few other ethnic groups such as Hausas, Igbos other non-Nigerians. The town hosts foreigners on daily basis due to the presence of Osun-Osogbo groove recognized internationally as a tourist centre. The town is blessed with primary, secondary, tertiary, and comprehensive

healthcare centres which make it advance the status of a semi-urban centre. Snails, Cercaria and Mice were used for the study.

2.1.1 Collection of snails

A total number of 150 snails were collected from a stream in Ilie Town, Osun state Nigeria. The snails were identified using individual morphological characteristics and structural differences from each other.

2.1.2 Cercarial shedding

The collected snails were exposed to light for an hour while a drop of water from which the snails were placed was examined under the microscope for the presence of cercariae. Following this step, the snails were carefully brought back to their respective aquarium tanks. The cercariae concentration was evaluated by counting their number in three aliquots of 0.5ml. The average of three counts was evaluated and taken as the number of cercariae in 0.5ml of the suspension. Hence, the number of cercariae in a known volume of a particular suspension was determined

2.1.3 Infection of mice

In this study, adult healthy mice weighing 20-25 g were fed normally (guinea feed) and clean portable water was used throughout the experiment. These animals were handled according to laboratory recommendations. In order to infect mice with the cercariae of *S. mansoni*, they were first induced to defaecate by passing them through lukewarm water in a bucket for about 30mins. The mice were then taken to infecting jars with perforated covers which contained 15 mls of lukewarm water (28-30°c). The paddling technique [13] was used for mice infection. Between 150 and200 cercariae were introduced into each jar through a calibrated Pasteur pipette. The animals were left to stand in water for an hour, thus allowing subcutaneous penetration of cercariae. Mice were then transferred back to their cages at the end of the infection period. The infection matured after 6 weeks post cercarial exposure

2.1.4 Treatment of infected mice

Three dosing protocols of combined medications were adopted intragastrically using a stomach tube. The treatment of the infected mice began on day 46 after infection, A total of 40 mice infected with *S. mansoni* were shared into three experimental groups (groups i, ii, and iii) including one untreated control group. (n=10 in each group).

2.1.5 Treatment protocol

Two-day protocol, Artemisinin 400 mg/kg + Praziquantel 500mg/kg daily
Four-day protocol, Artemisinin 200 mg/kg + praziquantel 250 mg/kg daily
Six-day protocol, Artemisinin 100 mg/kg + pranziquantel 125 mg/kg.

NB: No side effect was noticed. There was no noticeable significance in the amount of male or female *S. mansoni* worms found by perfusion of mice

2.1.6 Worm recovery

At the eight-week post cercarial exposure, the animals were sacrificed by cervical dislocation. The intestine and liver were placed in separate petri dishes and the worms were retrieved in physiological saline preparation. The number of parasites retrieved from each organ was counted, sexed and recorded.

Fragments of liver and small intestinal were processed one by one by the potassium hydroxide digestion technique for counting *S. Mansoni*e ggs in tissues (Cheever, 1968) [14]. Changes in oogram patterns in the mucosa of the small intestines of treated mice groups were studied and in comparison with the infected untreated control [15].

Liver sections were prepared and stained using haematoxylin and eosin (H & E) to examine hepatic granulomas including their healing in the treated groups compared to the infected untreated control.

Data collected from the study were presented as mean ± SD, analyzed by Analysis of variance (ANOVA) to evaluate the significance of differences observed among various experimental groups using Statistical Package for Social Science (SPSS) version 21 (SPSS Inc., Chicago, USA). P-value less than 0.05 ($p < 0.05$) was considered statistically significant.

3. RESULTS

Out of the 150 snails collected, 60 were confirmed to be *Biomphalaria* while the remaining 50 and 40 were *Bulinus* and *Intercalatum* respectively. Snails of the

*biomphalaria*s that were infected with miracidium of *Schistosoma mansoni* were included in the study (Table 1). In comparing worm reduction rate between the test and control groups as shown in Table 2, Artemisinin–Praziquantel combination caused significant total worm reductions of 58.2% in group I, 57.7% in group II while 43.9% was observed in group III as against the control. Also, there were significant worm reductions of 63.7% and 57.5% in groups I and II, respectively. However, group III demonstrated a lower worm burden reduction of 42.4% when compared with the control. Figs. 1-2 show schistosomes recovered from the mice. In comparing egg reduction rate in test and control groups as observed in Table 3, Artemisinin-Praziquantel combination induced significant reductions in eggs per gram liver tissue when compared with the control. Highest reduction of 66.3% was found in group II, compared with 58.8% and 56.5% significant reductions in groups I and III, respectively. Also, there were 77.8% and 74.2% significant reductions in eggs per gram of the small intestinal tissue noted in groups I and II respectively (p=0.0039) as against 63.1% reduction in group III. A significant decline in the percentage of total immature stages of 6.14% in group I was observed when compared to 66.14% in the control.

Additionally, a statistically significant increase of 57.57% was found in the protocol of Artemisinin 100 mg/kg + Praziquantel 125 mg/kg daily (6-day protocol) resulting in reductions of only 43.9% and 42.4% in the total and female worm burdens, respectively. Furthermore, the combination of Artemisinin-Praziquantel resulted in a significant reduction in tissue egg loads. Eggs in the wall of the small intestine were observed to be more affected by all the three combination dosing protocols than eggs retained in liver tissue. This combination brought down intestinal tissue egg loads ranging between 63.1%– 77.8% and liver egg loads in the range of 56.5%–66.3%. A reasonable alteration in the oogram pattern was also induced with a significant reduction in the total immature stages and a boost in the percentages of dead eggs (Table 4). The two-day protocol of Artemether 400 mg/kg + artesunate 500 mg/kg daily (2-day protocol) was the most efficacious in changing the oogram pattern, resulting in a decline in rate to 6.14% in total immature eggs by inducing the greatest increase in the percentage of dead eggs to 57.57%. The liver parenchyma was loaded with schistosomal granulomas surrounding newly laid eggs which were in multitude in the controls (Fig. 1). These were either active consisting of a central ovum bounded by epithelioid cells, lymphocytes, eosinophils, and a few giant cells or healed in which the inflammatory cellular infiltrate was changed by fibrosis (Fig. 2). Only group II demonstrated significant reduction of hepatic granulomas.

Table 1. Effect of different Artemether-artesunate protocols on worm burdens in *S. mansoni* infected mice

Group (n)	Dosing protocols	Total worms (mean ±SE)	Total worm reductions %	Total female worms (mean ±SE)	Total female worm reductions %
Control	Untreated	20.57±1.21	N.A	7.71±0.84	N.A
I	Two-day protocol	8.60±1.29	58.2	2.80±0.58	63.7 P>0.05
II	Four-day protocol	8.71±2.16	57.7	3.208±0.78	57.5 P>0.05
III	Six-day protocol	11.51±2.08	43.9	4.44±0.93	42.4 P>0.05

Note: NA means not applicable

Table 2. Effect of different Artemether-artesunte protocols on egg load reductions in *S. mansoni* infected mice

Group	Dosing protocols	Liver $\times 10^3$	Reduction %	intestine$\times 10^3$	Reduction %
Control	Untreated	16.51±1.69	NA	18.57±1.94	NA
I	Two-day protocol	6.80±0.66	58.8	4.80±1.02	74.2
II	Four-day protocol	5.57±0.90	66.3	4.13±0.67	77.8
III	Six-day protocol	7.19±0.74	56.5	6.86±0.69	63.1

Table 3. Effect of different Artemisinin-praziquantel combinations on oogram patterns in *S. mansoni* infected mice

Group	Dosing protocol	Total immature (mean ±SE)	Mature (mean ±SE)	Dead (mean ±SE)
Control	Untreated	66.14±3.55	28.71±4.01	5.15±0.74
I	Two-day protocol	6.14±0.75	36.29±2.50	57.57±2.97
II	Four-day protocol	30.50±2.96	33.00±2.02	36.50±2.77
III	Six-day protocol	23.77±3.35	29.44±5.05	46.79±5.41

Table 4. Effects of Artemisinin-praziquantel combination protocols on granuloma characteristics in experimentally infected mice which harbour adult *S. mansoni*

Group	Dosing protocol	Granuloma count/LPF (mean ±SE)	Reduction (%)	Granuloma diameter(µm) (mean±SE)	Reduction %	Healing ratio
Control	Untreated	23.00±1.27	NA	270.73±17.47	NA	1:2
I	Two-day protocol	16.60±2.31	27.8	217.70±12.18	19.6	2:1
II	Four-day protocol	11.00±0.71	52.2	207.10±17.71	23.5	2:1
III	Six-day protocol	17.40±2.56	24.3	199.20±12.50	26.4	5:1

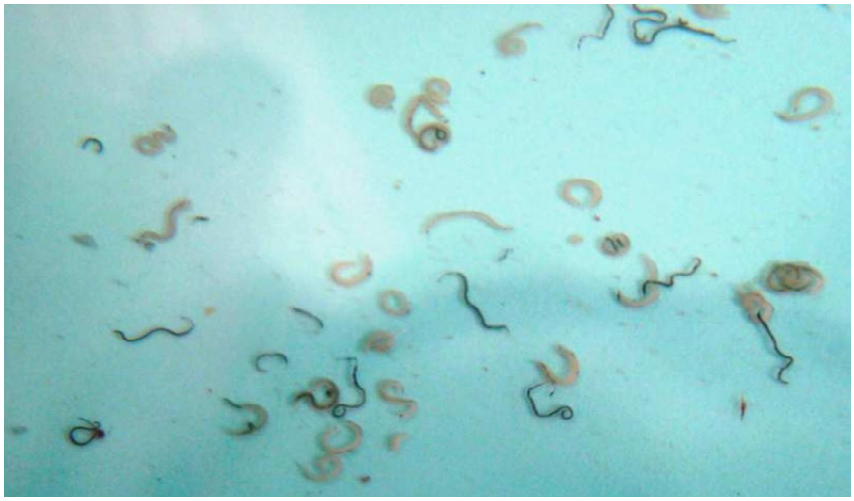

Fig. 1. Pinkish-colored male and dark-colored female *S. mansoni* worms recovered by perfusion of mice (actual size)

4. DISCUSSION

Artemisinin as an antimalarial derivative has been demonstrated as antischistosomal compounds with well-tolerated therapeutic dosage range and very effective. The drug has also been proven to be effective with low toxicity compared to many artemisinin derivatives particularly when administered orally and very safe because of its fast absorption and elimination [16]. This study investigated the *in vivo* therapeutic efficacy of combining Artemisinin and Praziquantel in mice infected with *S. mansoni*. In this study, the two-day protocol of Artemisinin 400 mg/kg daily including the four-day protocol induced the greatest reductions of >50% in total and female worm loads. The decline in adult worms in this study could be attributed to the fact that Artemisinin may be generally active against juvenile *S. mansoni* worms while adult worms are somewhat susceptible to it. This is in concordance with the study carried out by Sayed et al. [17] and the reduction of total adult worms with Praziquante could be as a result of the fact that the drug has effect mainly on adult *S. Mansoni* worms.

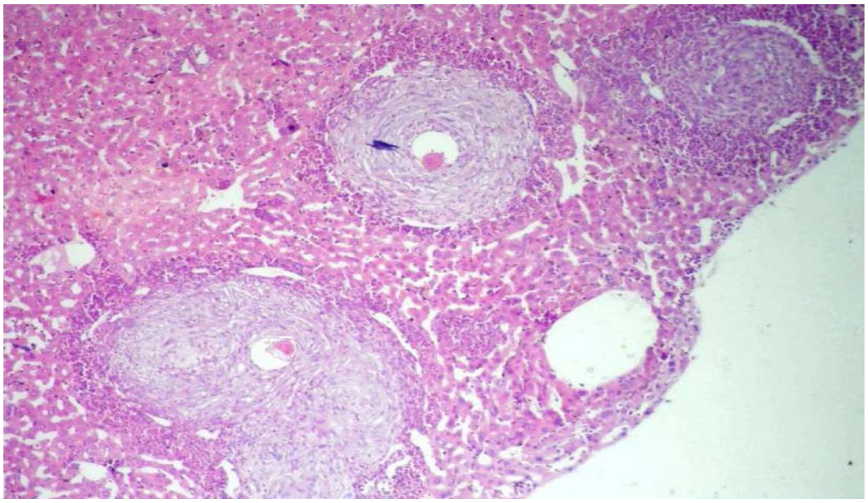

Fig. 2. Liver section from a treated group demonstrating one healed and two active hepatic schistosomal granulomas (H&E, ×40)

Nevertheless, the six-day protocol of Artemisinin100 mg/kg + Praziquantel 125 mg/kg adopted daily brought about reductions of only 43.9% and 42.4% in the total and female worm burdens respectively. Our study is in concordance with the report of Xiao et al. [18] which opined that egg count reduction is as a result of the effect of inhibition of artemether on sexual maturation leading to atrophy of worms' testis and ovaries. Also, the in egg load reduction in artesunate-treated groups can be elucidated by the fact that artesunate rectified the reproductive organs of *S. mansoni* female worms by reducing the ovarian volume and vitel-line follicles rarefaction [19]. Nevertheless, the leftover worms bounced back after artemether treatment became infertile and inability of laying eggs [20]. It therefore supports the fact that artesunate alters the fecundity of adult female worms instead of affecting their number.

Furthermore, the combination of Artemisinin–Praziquantel resulted in significant decrease in tissue egg burdens. Eggs in the wall of the small intestine are shown to be more pretentious by all the three combination dosing protocols than eggs left in liver tissue. This combination decreased intestinal tissue egg loads ranging between 63.1% to 77.8% and liver egg loads at a range of 56.5%–66.3%. A moderate change in the oogram pattern was also induced with a significant decline in the total immature stages and an increase in the preponderances of dead eggs. These results are in concordance with those of previous reports [21,22] The two-day protocol of Artemisinin was the most effective in changing the oogram pattern, causing a decline to 6.14% in total immature eggs and inducing the greatest increase in the preponderance of dead eggs to 57.57%. The other two protocols demonstrated lower effects on oogram pattern with the occurrence of all developmental egg stages of which there was still a significant ovicidal effect with the percentage of dead eggs increased to 36.50% and 46.78% when compared to 5.10% for the control. As a result of the alteration of oogram as noted, the findings of our study is comparable to earlier reports [21,23].

The four-day protocol of Artemisinin 200 mg/kg adopted daily in this study, was the most effective protocol for reducing hepatic granulomas. However, the highest healing ratio was accomplished when the combination was adopted with the use of six-day protocol. The amount of healed granulomas was five times higher than the active ones (5:1) when compared with a ratio of 1:2 for the control. On the other hand, the two-day protocol of Artemisinin 400 mg/kg + praziquantel 500 mg/kg daily in addition to the four-day protocol of artemisinin200 mg/kg + Artesuna 250 mg/kg daily resulted in the healed granulomas to increase twice the amount of the active ones (2:1) when compared to a ratio of 1:2 for the control. This could be explained by the increased treatment period as a result of a rapid elimination time of artemether. This is however contrary with earlier findings [21,24].

Moreover, previous studies [13,15] demonstrated that a single combined treatment with small doses of praziquantel and artemisinin was more

effective with 14- and 21-day-old schistosomula and 49-day-old adult *S. mansoni* (Liberian strain). The investigators found that the combination of the two medications simultaneously brought about a total worm decrease of 77% compared to only 2% and 66% with single administration of Praziquantel and artemether respectively [13,18,25]. Also, a female worm decrease of 85% was achieved with the combination when compared to 12% and 81% with Praziquantel and artemether respectively as reportted by Abdul-Ghani *et al.* [26]in their study.

5. CONCLUSION AND RECOMMENDATIONS

This study found highest worm load decline in 2-day protocol, artemisinin400mg/kg and Praziquantel 500mg/kg in addition to.highest egg reduction in 4-day protocol, artemisinin 200mg/kg + praziquantel 250mg/kg. Hence, combined effect of the drugs showed some level of efficacy on experimental *S. Mansoni*. It is nevertheless opined that further investigations on the efficacy of Artemisinin and Praziquantel combinations on *S. Mansoni,* putting into consideration both schistosomula and adult parasites are recommended. The result of such investigations will help in assessing the efficacy of the combined medications much more than the present study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical Approval was therefore sought from Animal Care and Use Research Ehics Committee (ACUREC), University of Ibadan, Nigeria for ethical clearance. The following procedures and steps were taken into consideration.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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