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Original Article

Evaluation of the Efficacy of Pediatric Suspension of Praziquantel against *Schistosoma mansoni* in Experimental Animals

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Abstract

Background: Schistosomiasis is one of the neglected tropical diseases with recent evidences about the high prevalence among preschool-age children. The pediatric formulation of Praziquantel (PZQ) has to be assessed for the efficacy as it gave controversial results in several countries.
Objective(s): The current study aimed at evaluating the efficacy of the pediatric suspension of PZQ against *Schistosoma mansoni* Egyptian strain in the experimental animals.
Methods: 150 Swiss albino mice infected with *Schistosoma mansoni* were divided into three groups, the first group was treated with 600 mg/kg body weight of PZQ pediatric suspension, the second group was treated with 600 mg/kg PZQ tablets and the third one received no treatment as a control. The efficacy of the pediatric formulation was experimentally evaluated in comparison with the tablet formulation as a benchmark on the basis of the following specific parasitological parameters (worm burden, tissue egg load, and oogram pattern i.e. percentage of dead, live or immature eggs shown in the stool sample).
Results: The comparison between the mean egg count per gram stool in the two groups pediatric suspension of PZQ (Epiquantel) and adult tablets of PZQ (Distocide), and the control group by applying one way ANOVA revealed a statistically significant difference ($p<0.05$) between the mean egg count in both treated groups (Epiquantel&Distocide) and their control group. The reduction of the total worm burden caused by Epiquantel®was96.9%, while that of Distocide®was86.7%, they were found to be statistically significant($p<0.05$) in comparison with the control group. Epiquantel® reduced the male worms by 100% and the females were reduced by 94.1%. Distocide showed a similar effect, it reduced the worms by 88.4% and 85.1% for males and females respectively. The administration of a single oral dose of both Epiquantel® and Distocide® resulted in a statistically significant reduction ($p<0.05$) in the mean egg count per gram tissue either the liver or the wall of small intestine when compared to their infected untreated control group. Complete absence of immature egg stages, high reduction in the mature eggs, and the increase in the dead eggs were observed in both Epiquantel® and Distocide® groups when compared to the control group.
Conclusion: The results prescribed that the pediatric suspension formula of PZQ is as efficient as the tablet formula against *Schistosoma mansoni* (Egyptian CD strain) in the mouse model. It could be recommended for pediatric treatment.

***Keywords*:** praziquantel, pediatric formula, experimental animals, *Schistosoma mansoni*

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INTRODUCTION

Human schistosomiasis or bilharziasis is a tropical parasitic disease caused by blood-dwelling fluke of the genus *Schistosoma*.[(1)] Schistosomiasis is endemic in 76 countries of Africa, Asia, and South America and considered to be one of the neglected tropical diseases. People at risk of schistosomiasis are estimated to be 779 million.[(2)] Because of unavailability of schistosomiasis vaccine, and the obstacles that face transmission control; the global strategy of schistosomiasis control depends on patients treatment. The latter using safe and effective antischistosomal drugs as recommended by the WHO Expert Committee against the three main species infecting humans.[(3)] Treatment mainly directed to risk groups (school-age children, adolescent and those whose occupations involve contact with infested water).[(4)] Praziquantel (PZQ) is the drug of choice for the treatment and morbidity control of schistosomiasis in many endemic areas in the world. It is highly effective against all major human schistosome species that cause severe intestinal

and urinary disorders, well tolerated and easy to be administered as a single oral dose.[5]

Praziquantel treatment has health benefits in preschool-aged children exposed to schistosomiasis and its efficacy on infection and morbidity is not age-dependent but the lack of appropriate pediatric formulations of PZQ resulted in their exclusion from treatment in population-based control programs for a long time.[6] This creates a potential health inequality. Several studies showed that children carry the heaviest burden of infection in affected populations, with documentation of high prevalence rates of schistosomiasis among preschool-age children (1-5 years).[7] In areas of Mali, Niger, Sudan, Uganda and Zimbabwe, the prevalence of the infection ranged from 18% to 63%.[8]This necessitates their inclusion in schistosome control programs.[9] The Schistosomiasis Research Project (SRP) has developed a pediatric formula of PZQ (Epiquantel®) suitable for young children and others unable to swallow the large and very bitter PZQ tablets. This suspension is being marketed by the Egyptian International Pharmaceutical Industries Company (EIPICO). The efficiency of this formula was tested among young children in Egypt, Mali, Uganda and Niger but controversial results were obtained.[10]

The aim of this study was to evaluate the efficacy of the pediatric formula of PZQ against *Schistosoma mansoni* Egyptian strain in the experimental animals using PZQ tablets as a benchmark.

METHODS

An experimental infected- control animal study design was conducted between November, 2011 and April 2012. It has been carried out in parasitological lab and animal house of High Institute of Public Health.

Experimental animals(Mice): Eight-week-old female CD-1 *Swiss albino* mice, weighing 20±2 g, were purchased from the Schistosome Biologic Supply Center (SBSC), Theodore Bilharz Research Institute (Giza, Egypt). Mice were kept under environmentally controlled conditions (temperature: ~25°C; humidity: ~70%) at the animal house at the High Institute of Public Health (Alexandria, Egypt). They had free access to water and food.

Animal infection with *S. mansoni*: In this experiment we used cercariae of *S. mansoni* (Egyptian/CD strain), maintained in laboratory-bred *Biomphalaria alexandrina* snails. Mature laboratory bred *Biomphalaria alexandrina* snails (50 snails) infected with *S. mansoni* (Egyptian/CD strain) were purchased from SBSC, TBRI two days before the infection of mice. Snails were kept in a dark place in a glass jar containing aged dechlorinated tap water and fresh lettuce leaves were provided as food. At the day of infection, snails were transferred to an empty and clean beaker and left under an electric lamb to induce cercarial shedding for 20 minutes. Then 20 ml of declorinated water were added to the beaker and left for additional 30 minutes. After gentle mixing of water, three aliquots 50µl each were taken on a microscopic slide and a drop of iodine was added for fixation and staining of cercaria. [11] The number of cercariae per each 50µl was counted under low power microscope (x5) and the mean number per 1ml of water was calculated to be sure that the number of cercariae was enough to infect 150 mice. After that snails were returned back to the jar and a common pool of cercariae was ready for the infection of mice. Each mouse was infected with 80 cercariae using the body immersion technique.[12]

Experimental groups: The infected mice were randomly divided into three groups. The least allowed number of alive experimental animals has to be 10 for each group. We started with 50 expecting some of them to die during the experiment.

i) **Epiquantel group**: The infected mice received a single dose of Epiquantel® suspension 49 days post-infection.

ii) Distocide group: The infected mice received a single dose of Distocide® 49 days post-infection.

iii) Control group: The infected mice received no treatment.

Drug therapy: Two different formulations of PZQ (Epiquantel® suspension and Distocide® tablets) were used in this experiment. They are manufactured by the Egyptian International Pharmaceutical Industries Company (EIPICO). **Epiquantel®** is available in bottles containing 15 ml suspension, equivalent to 1800 mg active ingredient (Batch No. 087684). It was administered directly to the mice intragastrically using a stomach tube in a single dose of 600 mg/Kg body weight, 49 days post-infection. **Distocide®** is available as tablets containing 600 mg of PZQeach (Batch No. 1100376).It was used as a freshly prepared suspension in 6% Cremophore EL directly before administration to the mice intragastrically using a stomach tube in a single dose of 600 mg/Kg body weight, 49 days post-infection. [13]

Indices for the evaluation of chemotherapy:

Parasitological assessment of PZQ formulations efficacy: Fecal samples were collected and weighed from each group of mice over a two-hour period on the 48th and 49th days post-infection before administration of the drug. After drug administration fecal samples were collected starting on the 50th day post-infection and then every other day until the end of the experiment (two weeks after treatment). Stool samples were collected for all mice in each group. Collected stool samples were suspended in about 10-15 ml saline comminuted well, sieved and centrifuged at 1000 rpm for 5 minutes. Supernatant was decanted the sediment was suspended in 2-4 ml saline. Before microscopic examination, the sediment was vortexed, six 50µl aliquots were examined microscopically with high power (x40) and *S. mansoni*

ova were identified and counted. The number of eggs per gram (epg) of feces was calculated by multiplying the mean number of eggs in 50 µl sample by the total volume of the saline suspension and dividing this value by the weight of the sample in grams, [14]

Estimation of worm burden: Mice in the three groups (Epiquantel®, Distocide® and infected untreated control group) were sacrificed by cervical dislocation 63 days post-infection (two weeks after drug administration). Worms were recovered from the hepatic and portomesenteric veins using Smithers and Terry perfusion technique for mice.[15] The small and large intestines were placed in a Petri dish, and all *S. mansoni* in the mesenteric veins were removed. The recovered worms were transferred into a Petri dish to be counted and sex differentiated under a stereomicroscope.

Oogram pattern: The small intestine was separated and transferred to a Petri dish. Three pieces each 1cm in length of the small intestine were cut and opened longitudinally, rinsed in saline, slightly dried on filter paper, and then compressed between two slides. The fragments were examined by low- power microscopy. A total of 100 eggs per animal were observed, and the stage of each egg. The egg in the first stage has a small embryo, of about one third of the transverse egg diameter. When the embryo becomes slightly larger than half the transverse egg diameter, the immature egg is considered to be in the second stage; and when it is two-thirds of the longitudinal diameter of the egg, it is in the third stage. In the fourth stage the embryo occupies the whole of the egg shell.[11]

Tissue egg load: The number of eggs/gram of tissue was determined by weighing 0.3g of liver and small intestine from each mouse and was digested overnight in 5 ml potassium hydroxide (KOH) (5%), after complete digestion, the samples were mixed well by vortex and 3 aliquots of 100 µl each were examined microscopically under low power (x5). The hepatic and intestinal tissue egg loads (expressed as epg) were determined by multiplying the number of eggs in each 100-µl sample by the total volume of KOH and dividing this value by the weight of the sample in grams.[15]

Statistical analysis: Statistical analysis was performed using SPSS (Statistical Package for the Social Sciences) version 17.0 for Windows®. Drug efficacy was assessed by the comparison between the three groups using independent one way ANOVA. Differences were considered statistically significant when *p*-values were ≤ 0.05. The comparison between the treated groups (Epiquantel & Distocide) and their control using repeated measures ANOVA (Analysis of Variance) followed by Tukey's HSD post hoc test.

Ethical Considerations

The study was approved by the institutional review board, and ethics committee of the High Institute of Public health - Alexandria University, Egypt. Scientific procedures and guidelines for the care and use of animals were followed. Laboratory animals were reduced; the minimum number of mice was used to obtain scientifically valid results. Appropriate feeding was ensured for the welfare of animals. An appropriate euthanasia method was used to produce rapid loss of consciousness without pain until death occurs.

RESULTS

The comparison showed that the treated groups (Epiquantel & Distocide) were statistically significant different (F-test, 15.1; p<0.05) to control group. The interaction between groups was insignificant (F-test, 3.5; p= 0.07). The repeated measures ANOVA revealed no statistically significant difference between the two treated groups (p>0.05). Repeated measures ANOVA revealed a statistically significant difference (F-test, 36.7; p<0.05) between mean egg count per gram stool over all time periods (before and after treatment) (Table 1).

Table 1: Effects of two formulations of PZQ (Epiquantel®&Distocide®) administered 49 days post infection on mean egg count per gram stool in mice harboring adult *S. mansoni* (Egyptian CD strain)

Group	No. of mice	Egg count/ Phase					
		Before treatment		One week after		Two weeks after	
		Mean	SD	Mean	SD	Mean	SD
Epiquantel	**16**	5924.5	2906.2	3374.5	1068.3	213.1	2.2
Distocide	**15**	7337.8	654.9	3550.8	849.3	538.1	222.4
Control	**8**	9649.5	117.4	6274.5	994.2	7274.8	2548.2
P for interaction	F=3.5; P = 0.071						
P for treatment	F=15.1; P = 0.014[*]						
P for time	F=36.7; P = 0.001[*]						

P: P value based on repeated measures ANOVA. F: Fisher Exact Test

- [*] P < 0.05 (significant).
- Control was significantly different of treated groups.
- All time periods were significantly different.

Table (2) demonstrates that Epiquantel® induced a significant higher reduction (96.9%) in the total worm burden than that caused by Distocide® which was 86.7%, in comparison with control group. The difference between both reductions was found to be statistically significant ($p<0.05$). Male worms were more sensitive to the two formulations of drug than females. Where Epiquantel® reduced the male worms by 100% and the females were reduced by 94.1%. Distocide® showed a similar effect on the worms but with lower efficiency, it reduced the worms by 88.4% and 85.1% for males and females respectively. There was a statistically significant difference ($p<0.05$) between Epiquantel® and the control group as regards the distribution of worms in the portomesenteric vessels and in the liver. The percentage of portomesenteric worms in the control group was higher (88.8%) than those of Epiquantel®, which exhibited percentage of 70.0%. On the other hand, the control group percentage of hepatic worms was 11.3%, lower than Epiquantel® treated group (30.2%). The percentage of portomesenteric worms in the control group was higher (88.8%) than those of Distocide® which exhibited percentage of 55.0%, the difference between portomesenteric worms in the two groups was statistically significant ($p<0.05$). The difference between the hepatic worms in Distocide® group and the control group was found to be statistically insignificant ($p>0.05$), the percentages of the hepatic worms were 44.9% &11.3% in Distocide® group and control group respectively. The comparison between Epiquantel® and Distocide® showed a statistically significant difference ($p<0.05$) as regards the distribution of worms in the portomesenteric vessels and in the liver. Where Epiquantel® had higher percentages than the corresponding ones of Distocide®. The administration of a single oral dose of Epiquantel® suspension resulted in a statistically significant reduction ($p<0.05$) in the mean egg count per gram tissue of either the liver or the wall of small intestine when compared to the infected untreated control group. The reduction in the liver egg load was 47.28%; while it was 79.74% in the wall of small intestine. Distocide® was found to induce reductions in the eggs per gram tissue in both the liver and the wall of small intestine that were (35.56% & 75.21%) respectively, these reductions were statistically significant ($p<0.05$) in comparison with the control group. There was no significant difference ($p>0.05$) between Epiquantel® and Distocide® (Table 3).

Table 2: Effects of two formulations of PZQ (Epiquantel®&Distocide®) administered 49 days post infection on worm burden, sex and distribution in mice harboring adult *S. mansoni* (Egyptian CD strain)

Group	Mean number of worms ± SD					(%)				
	Hepatic worms	Intestinal worms	Total males	Total females	Total worms	Hepatic worms	Porto-mesentric worms	Total worm burden reduction	Female worm reduction	Male worm reduction
Epiquantel (n=16)	0.2±0.5 [*][a,b]	0.4±1.1 [*][a,b]	0.0±0.0	0.6±1.4	0.6±1.4 [*][a,b]	30.2	70.0	96.9	94.1	100
Distocide (n=15)	1.2±1.6	1.5±1.6 [*][a]	1.1±1.2	1.6±1.99	2.7±2.4 [*][a]	44.9	55.0	86.7	85.1	88.4
Control (n=8)	2.3±1.5	17.8±7.9	9.3±3.4	10.8±4.2	20.0±7.0	11.3	88.8	---	---	---

No. of mice/group is given in parentheses.
[a]: Compared to infected untreated control group.
[b]: Compared to Distocide group.
The treated groups were tested versus each other and versus control group using independent-samples t-test; [*]: $p\leq0.05$.

Table 3: Effects of two formulations of PZQ (Epiquantel®&Distocide®) administered 49 days post infection on tissue egg load in mice harboring adult *S. mansoni* (Egyptian CD strain)

Group	No. of mice		Tissue egg load (epg)			
			Liver (mean epg ±SD)×10^{-3}	**Reduction (%)**	**Intestine** (mean epg ±SD)×10^{-3}	**Reduction (%)**
Epiquantel	16	16	13.8±4.8 [*][a]	47.3	11.1±5.47[*][a]	79.7
Distocide	15	15	16.8±5.3 [*][a]	35.7	13.6±6.8 [*][a]	75.2
Control	8	8	26.1±6.8	----	54.7±10.7	----

- The treated groups were tested versus each other and versus control group using independent-samples t-test
[*]: $p\leq0.05$.
-[a]: Compared to infected untreated control group.

As regards the decrease in the percentages of the total immature egg stages, it was found that there was a statistically significant difference (p<0.05) between the two formulations of PZQ(0.00% and 0.01% in Epiquantel & Distocide, respectively) and the control group (35.8%). In addition, there was a significant increase in the percentage of dead eggs in both groups (68.06% in Epiquantel group and 54.39% in Distocide group) than the control group (13.99%) (p<0.05). The percentage of mature stage was highest in control group (50.22%), whereas it showed a significant reduction in the Epiquantel group (31.94%) (p<0.05) & and insignificant reduction in the Distocide group (45.59%) (p<0.05). The comparison between Epiquantel & Distocide revealed that there was a significant difference (p<0.05) in the total immature stages of the two groups, while the mature stage and the dead eggs showed no significant difference (p>0.05) (Table 4).

Table 4: Effects of two formulations of PZQ (Epiquantel®&Distocide®) administered 49 days post infection on oogram pattern in mice harboring adult *S. mansoni* (Egyptian CD strain)

Group	No. of mice	Developmental stages of egg (mean±SD)		
		Total immature	Mature	Dead
Epiquantel	16	0.0±0.0 [*][a]	31.9±12.2 [*][a,b]	68.1±12.2 [*][a,b]
Distocide	15	0.0±0.1[*][a]	45.6±16.3	54.4±16.3 [*][a]
Control	8	35.8±8.5	50.2±9.3	13.99±6.9

The treated groups were tested versus each other and versus control group using independent-samples t-test;
[*]: $p \leq 0.05$.
[a]: Compared to infected untreated control group.
[b]: Compared to Distocide group.

DISCUSSION

Our main finding in experimental animals supports the studies reported that the suspension formula of PZQ is as effective as the tablet formula in the treatment of schistosomiasis in young children. There were conflicting results about the efficacy of the pediatric formula of PZQ (Epiquantel®) in the treatment of schistosomiasis in children reported from different countries. While two studies reported cure rates similar to that of tablet formula, another two studies reported low cure rates.[9,10] The first finding of our study was the continued decrease in the number of eggs per gram stool in the two treated groups after drug administration. The statistically significant difference between the treated groups and their control were due to the effect of the drugs either by killing the adult worms or alteration of oviposition.

The present study showed that, Epiquantel® significantly reduced the worm burden of S.*mansoni* (Egyptian CD strain) by 96.88%, as compared to both the infected untreated control group and the group treated with Distocide®. Similar results were obtained by Keiser *et al.* (2006) reported that the oral administration of a 500 mg/ kg of Distocide® to male *Swiss albino* mice that were infected with 80 *S. mansoni* cercariae (Egyptian/CD strain) 49 days PI resulted in a 92.9% reduction in the total worm burden.[16] Also,in two separate studies performed under the same conditions of the present study, where female *Swiss albino* mice were infected with 80 *S. mansoni* cercariae (Egyptian/CD strain) and treated with a single oral dose of Distocide® 49 days PI, the authors reported nearly similar results, 89.9% and 83.4% reduction in total worm burden respectively. [17,18] A hepatic shift of the worms was observed to be caused by both Epiquantel® & Distocide® where a 70%& 55% of the worms were collected from the portomesentric region compared to 88.75% in the control group. These results are considered low with regard to the results of previous studies where a very high hepatic shift was observed (21%-29.4% of the worms were collected from the portomesentric region). [16-18] Epiquantel® was found to target the male worms of the Egyptian CD strain of *S.mansoni* more than the females. Extreme antischistosomal activity with a very high reduction of female worms by 94.1% and a complete reduction of male worms by 100% (no male worms were recovered) was observed. On the other hand, Distocide® also was found to affect males more than females. Previous studies showed controversial results of the effect of PZQ on the sexes of *Schistosoma*. Some showed that there was no preferential effect of PZQ on either sex, other studies showed that PZQ tend to affect female adult worms more than adult males.[16-19] The reduction in the tissue egg loads in the liver and the small intestine induced by Epiquantel® was found to be insignificantly higher than that of Distocide®. The findings of the present study were in agreement with the findings of the study of Delgado *et al*. where they found the average reductions of the number of eggs per gram liver to be 53.3% in three different strains of *Schistosoma* treated with 500mg/kg PZQ in the mouse model. However, the

intestinal egg loads in the same study were found to be somewhat higher (89.7%).[19]

As regards oogram pattern, Epiquantel® had a significant alteration in the oogram pattern. The comparison between Epiquantel® and Distocide® revealed that there was a significant difference in the total immature stages of the two groups (Epiquantel® was more efficient than Distocide®), while the mature stage and the dead eggs showed no significant differences. Previous studies revealed higher alterations in the oogram pattern than the present study, where they reported an increase in dead eggs reached 79.6% of all stages. The mature eggs percentages were16.6%- 18.1% and the immature egg stages represented 3.9%-8.8%.[16-18] A recent RCT (randomized controlled trial) in 2017 concluded that, a single dose of PZQ of 40 mg/kg for *S mansoni* infections used in school aged-children (SAC) can be endorsed for PreSAC in preventive chemotherapy programmes in the absence of treatment alternatives.[20] The circulation of generic drugs of substandard quality in the developing countries might be the situation in those studies reported low cure rates of PZQ suspension formula.[21] PZQ works in synergy with the immune system of the human definitive host, sufficient immune mechanisms of those young children might not be developed at the time of treatment.[22]

CONCLUSION AND RECOMMENDATIONS

Epiquantel is highly effective in treatment of *Schistosoma mansoni* as assessed experimentally through parasitological parameters of its efficacy. The availability of a pediatric-friendly formulation of PZQ resolves the PZQ treatment gap and there is much optimism for African children to have better access to medication. We recommend the reassessment of the effectiveness of Epiquantel® in treating human infection with S. mansoni in the countries where it previously showed low cure rates accompanied with further pharmacokinetic studies to determine the optimal praziquantel dose for young children of different age stages.

Conflict of Interest: None to declare.

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