The Effect of Different Formulations of Praziquantel in Reducing Worms in the Prepatent Period of Schistosomiasis in Murine Models

Érica Tex Paulino 1,2,3, Monique Ribeiro de Lima 4, Alessandra Lisfitch Viçosa 5, Cleber Hooper da Silva 6, Claudio Javier Salomon 7,8, Daniel Andrés Real 7,8, Dario Leonardi 7,8, Clélia Christina Mello Silva 2* and Antonio Henrique Almeida de Moraes Neto 1†

1 Laboratory of Innovations in Therapies, Teaching and Bioproducts, Oswaldo Cruz Institute, Oswaldo Cruz Foundation (LITEB/IOC/FIOCRUZ), Rio de Janeiro, Brazil, 2 Laboratory of Environmental Health Evaluation and Promotion, Oswaldo Cruz Institute, Oswaldo Cruz Foundation (LAPSA/IOC/FIOCRUZ), Rio de Janeiro, Brazil, 3 Tropical Medicine Program, Oswaldo Cruz Institute, Oswaldo Cruz Foundation (IOC/FIOCRUZ), Rio de Janeiro, Brazil, 4 Animal Experimentation Center, Oswaldo Cruz Institute, Oswaldo Cruz Foundation (IOC/FIOCRUZ), Rio de Janeiro, Brazil, 7 Laboratory of Experimental Pharmaceutics, Farmanguinhos, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, 8 Laboratory of Experimental Pharmaceutics, Farmanguinhos, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, 6 Institute of Science and Technology in Biomodels, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil, 7 Faculty of Biochemical and Pharmaceutical Sciences, University of Rosario, Rosario, Argentina, 8 Institute of Chemistry of Rosario—National Research Council Scientific and Techniques (IQUIR-CONICET), Rosario, Argentina

Schistosomiasis is a widely distributed parasitic disease and one of the most important neglected tropical diseases globally, for which Praziquantel® (PZQ) is the only available treatment. In this context, tests with new PZQ formulations become relevant for disease control. This study evaluated the effects of PZQ treatment in the prepatent phase of schistosomiasis using two formulations: nanoencapsulated (PZQ-NANO) and active pharmaceutical ingredient (PZQ-API). Five experimental groups were established, for which the following serological parameters were evaluated: ALT, AST, ALP, and TP. Animals treated with PZQ-API at 15 and 30 days post-infection showed decreased eggs per gram of feces (EPG) compared to untreated infected animals. The same animals showed reductions of 63.6 and 65.1%, respectively, at 60 days post-infection. Animals treated with PZQ-NANO experienced no significant changes in EPG at any time of observation. Treatment with PZQ, either API or NANO, at 15 days post-infection reduced AST, ALT, and TP levels. It is concluded that prepatent treatment with PZQ-API can reduce the parasite load of infected animals and that treatment at 15 days post-infection can prevent increased serum levels of ALT, AST, and TP.

Keywords: schistosomiasis, Praziquantel (PZQ), prepatent infection, Schistosoma mansoni (S. mansoni), nanoencapsulated

INTRODUCTION

The World Health Organization (WHO) considers schistosomiasis to be one of the most important neglected tropical diseases. It affects more than 290 million people globally (1) and has chronic development that causes debilitating morbidity (2). This total, however, reaches ~440 million people when individuals cured of infection, but with remaining residual morbidity, are included (3).
According to the Brazilian Ministry of Health (4), 1.5 million Brazilians are at risk of contracting the disease because they live in endemic areas. Between 2020 and 2021, the period corresponding to the COVID-19 pandemic, 212 hospitalizations and seven deaths from schistosomiasis were registered in the country, with cases being more frequent in the Northeast and Southeast regions (5).

The current recommendation for the control of schistosomiasis is based on the use of Praziquantel® (PZQ), not only because of its action in reducing disease prevalence and morbidity, but also for its schistosomicidal activity (6). However, the characteristic pathogenesis of the disease, namely hepatic periovular granuloma, still remains even with treatment since eggs of *S. mansoni* are carried to other organs via blood flow (7).

Although several studies have demonstrated the efficacy of PZQ against adult worms, larval forms are insensitive to its chemotherapeutic action (8–11) due to its marked evolutionary asynchronism (12–14). A treatment with proven effectiveness in the prepatent period of schistosomal infection would significantly benefit human health, since without the development of adult forms there would be no egg production and, consequently, no formation of granulomas nor the development of other comorbidities (14).

Nanotechnology has recently been used as an effective strategy for drug delivery to predetermined targets. The method presents a series of advantages such as increased bioavailability, decreased systemic drug absorption, reduced side effects, accurate drug targeting, and increased time of action in the body, as the release of the bioactive agent is gradual and controlled (15–17).

The search for efficacy in treating mass infections, or for new formulations containing PZQ or even new drugs, becomes relevant to achieving adequate control of schistosomiasis in endemic areas. Thus, this study aimed to evaluate the effects of PZQ treatment in the prepatent phase of *S. mansoni* infection under experimental conditions using two different formulations: nanoencapsulated and active pharmaceutical ingredient only.

**METHODS**

Two-hundred and eighty female mice, *Mus musculus* (Swiss Webster strain), ∼4 weeks old (20–25 g), were kept, infected, and euthanized in a certified vivarium following IOC/FIOCRUZ bioethics and safety rules. All animals were maintained with a 12-h light/12-h dark cycle, humidity control, environmental enrichment and feed (previously autoclaved) and water provided ad libitum. Individual mice were experimentally infected subcutaneously with 0.3 ml of a suspension of 150 (± 10) cercariae (within 2 h after their release from snails) in dechlorinated water (18). The LE strain of *S. mansoni* used in this experiment came from the Schistosomiasis Reference Service of the Malacology Laboratory of IOC/FIOCRUZ. All animals were infected only once and followed until blood collection for serological exams.

The formulations of PZQ used were “pharmaceutical active ingredient” (PZQ-API) and nanoencapsulated (PZQ-NANO). PZQ-NANO was produced by nanoprecipitation, using a polymer (Eudragit E100) contained in an organic phase and PZQ in an aqueous phase containing a stabilizer to guarantee the use of this nanoparticle in various drug formulations (19, 20). Polyvinyl alcohol (PVA) was used as an anti-aggregating agent to prevent coalescence of nanoparticles. The obtained nanosuspension was then dried by “Spray Drying” using spray-drying equipment (Buchi B- 290), with Maltodextrin (dextrose equivalent 4.0–7.0) as a diluent, to obtain a stable formulation that guarantees stability during experiments. The qualitative and quantitative composition of the nanoparticles was 20.8% PZQ (0.208 mg PZQ in 1 mg of nanoparticulate material), 20.8% Eudragit E, 8.4% PVA, and 50% Maltodextrin. Both formulations used in this study were administered to mice in aqueous suspension via a single dose of 400 mg/Kg by gavage. The chosen dosage was based on studies attesting to its effectiveness in treating schistosomal infection in mice (21, 22).

The mice were randomly allocated to five experimental groups (Figure 1) and acclimatized for 15 days. Animals of the infected and untreated group (IN) and of the infected and treated group (INTR) were then infected, and those of uninfected and treated group (TR) and INTR were treated with PZQ-API or PZQ-NANO. Thus the five experimental groups were: G. IN, G. TR-API, G. TR-NANO, G. INTR-API and G. INTR-NANO. Observations were made at 15, 30, 60, and 90 days post-infection (p.i.) to analyze the behavior of the infection and treatment in the prepatent (15 and 30 days) and patent (60 and 90 days) stages of infection. Mice belonging to groups INTR-API and INTR-NANO were organized into four subgroups with 20 animals each, which were treated with PZQ (API or NANO, respectively) at 15, 30, 60, and 90 days p.i.

All serological tests were performed at the Technological Platform—Laboratory Animals/Clinical Analyses based at Institute of Science and Technology in Biomodels (ICTB/FIOCRUZ). The following biochemical parameters were analyzed: Alkaline Phosphatase (ALP; reference value: 62 to 209 U/L), Alanine Aminotransferase (ALT; reference value: 28 to 132 U/L), Aspartate Aminotransferase (AST; reference value: 59 to 247 U/L) and Total Proteins (TP; reference value: 3.6 to 6.6 g/dL). Blood collections were performed by cardiac puncture, using a disposable syringe with a 20 x 0.55 mm needle, of animals previously anesthetized with 100 mg/kg of ketamine associated with 10 mg/kg of xylazine hydrochloride via intraperitoneal, with anesthesia depth being verified by sensitivity test using painful stimuli. Collected blood was transferred to a 500-µl microtube with gel and clot activator and centrifuged at 13,000 RPM, for 3 mins, at room temperature. The serum was then transferred to a 0.5 ml Eppendorf and sent to the clinical analysis platform of ICTB/FIOCRUZ for serological analysis. Ten infected animals (G. IN) and ten animals treated with PZQ (G. TR-NANO and G. TR-API) were selected at each observation time (15, 30, 60, and 90 days p.i. or treatment) for blood collection. In the infected and treated groups (INTR-NANO and INTR-API), blood was collected from 10 animals, 24 h post treatment with PZQ, at the same observation times. Animals that did not die after this procedure were euthanized by barbiturate overdose: intracardiac administration of 2.5% sodium thiopental (300 mg/kg) by the...
FIGURE 1 | Flowchart of the experimental design of the study.

Female mice
*Swiss Webster*
(n=280)

Infected Group
**G. IN**
(n=40)

Infected and Treated Group
**G. INTR**
(n=160)

Treated Group
**G. TR**
(n=80)

Infection with *S. mansoni* cercariae (BH strain)
15 days after setting the mice

**G. INTR-API**
(n=80)

**G. INTR-NANO**
(n=80)

**G. TR-API**
(n=40)

**G. TR-NANO**
(n=40)

Treatment performed according to the time after infection

Subgroup 01 - 15 days (n=20)
Subgroup 02 - 30 days (n=20)
Subgroup 03 - 60 days (n=20)
Subgroup 04 - 90 days (n=20)

Treatment with PZQ API or PZQ NANO
15 days after setting the mice

Blood collection to assess parameters AST, ALT, ALP e TP

**G. IN** - after infection
- 15 days (n=10)
- 30 days (n=10)
- 60 days (n=10)
- 90 days (n=10)

**G. TR** - after treatment
- PZQ-API / PZQ-NANO
  - 15 days (n=10)
  - 30 days (n=10)
  - 60 days (n=10)
  - 90 days (n=10)

**G. INTR** - 24h after treatment
- PZQ-API / PZQ-NANO
  - 15 days (n=10)
  - 30 days (n=10)
  - 60 days (n=10)
  - 90 days (n=10)

Collection of feces to determine the parasitic burden of infected animals

**G. IN** - after infection
- 60 days (n=10)
- 90 days (n=10)

**G. INTR** - after infection
- PZQ-API / PZQ-NANO
  - 60 days (n=10) Subgroups 15 days,
  - 90 days (n=10) 30 days e 60 days
  - 90 days (n=20) Subgroup 90 days
responsible veterinarian, according to the protocol approved by the Animal Ethics Commissions (CEUA).

Parasitic load was determined as the number of eggs per gram of feces (EPG) using the Kato-Katz method (23). Feces were collected from infected mice at 60 and 90 days p.i. (prior to treatment for those that received treatment at 60 and 90 days p.i.). Feces were collected in the morning, individually and for an hour to avoid animal stress, after which mice were returned to their respective cages. Three slides were prepared for each feces sample. Counts were made under a light microscope and the arithmetic mean of eggs per feces sample was calculated and multiplied by 24 (factor) to obtain EPG. Parasitic load was classified according to the criteria adopted by the WHO (24), and referenced in the protocol of the Kato-Katz Kit, as follows: 1–99 EPG = light intensity; 100–399 EPG = moderate intensity; ≥ 400 EPG = heavy intensity.

The biochemical parameters of serological tests and the results of the EPG were compared between the different groups of both pharmaceutical formulations containing PZQ for the evaluation of the therapeutic responses of the mentioned formulations. The Mann-Whitney-Wilcoxon test was used to assess differences between groups treated with PZQ-NANO and PZQ-API, using the group of untreated infected animals (G. IN) and the group of treated and uninfected animals (G. TR-NANO and G. TR-API) as controls. All results were input into Microsoft Access database software and analyzed using R version 4.0.2 (25) with a significance level (α) of 5%.

RESULTS

In all, 120 feces samples were collected from animals at 60 days p.i. and 110 from animals at 90 days p.i.

Table 1 presents the results of the Mann-Whitney-Wilcoxon paired test for EPG at 60 and 90 days p.i. The IN control group and subgroups INTR-API 15 days, INTR-API 30 days and INTR-API 60 days showed significant decreases in EPG at 90 days p.i. The subgroups INTR-API 15 days and INTR-API 30 days, both treated in the prepatent period of infection, showed EPG reductions of 63.6% [1–(78.8/216.9)] and 65.1% [1–(75.6/216.9)], respectively, at 60 days p.i., when compared to the IN control group. This reduction was also observed at 90 days p.i., with the subgroups INTR-API 15 days and INTR-API 30 days showing EPG reductions of 72.3% [1–(27.6/92.4)] and 70.1% [1–(25.6/92.4)], respectively, compared to the IN control group. There was no significant difference in EPG for PZQ-NANO treated groups at both 60 and 90 days p.i., compared to the IN control group. However, animals treated with PZQ-API having a greater reduction in EPG at 90 days p.i. than animals treated with PZQ-NANO (Supplementary Figure 1).

Analysis of animals infected and treated with either PZQ-API or PZQ-NANO revealed significantly lower serum levels of AST, ALT and TP for subgroups INTR-API 15 days (Figures 2A,B,D) and INTR-NANO 15 days (Figures 3A,B,D). For infected and untreated animals, IN 15 days had significantly lower serum AST levels than IN 30 days (P < 0.01) (Figure 2A) and lower serum ALT levels than IN 30 days (P < 0.01) and IN 60 days (P < 0.05) (Figure 2B).

Comparison of groups infected and treated with either PZQ-API or PZQ-NANO for parameters ALT, AST, ALP and TP (Supplementary Figure 2) revealed that ALT and TP did not differ significantly between subgroups with the same treatment time but of different drug treatments (PZQ-API or PZQ-NANO) (Supplementary Figures 2B,D). There were lower serum AST levels for subgroup INTR-API 90 days compared to subgroup INTR-NANO 90 days (P < 0.01) (Supplementary Figure 2A), while serum ALP levels were significantly higher for subgroup INTR-API 15 days compared to subgroup INTR-NANO 15 days (P < 0.05) (Supplementary Figure 2C).

Analysis of serum levels of ALT and AST between the IN control group and groups INTR-API and INTR-NANO revealed a significant increase (P < 0.05) for animals treated during the patent period of infection, that is, 60 and 90 days p.i. (Figures 2A,B, 3A,B). Animals of the groups TR-API and TR-NANO maintained significantly lower serum levels of ALT and AST compared to groups INTR-API and INTR-NANO, respectively, showing that only treatment with the PZQ formulations evaluated in this study in uninfected animals did not related to increased serum levels of these enzymes (Figures 2A,B, 3A,B).

There were higher serum levels of ALP at 15 days p.i. and lower levels at 60 days p.i. for both IN (P < 0.05) and INTR-API (P < 0.01) (Figure 2C). The results remained within the reference range (62 to 209 U/L), except for two animals in the subgroup “INTR-API 15 days” (244 U/L and 219 U/L). Serum levels of this enzyme was increased in both IN and INTR-API at 90 days p.i., with three animals in INTR-API having values above the reference range (i.e., 690 U/L, 284 U/L and 268 U/L). Animals of INTR-NANO and TR-NANO did not differ in serum levels of ALP at any of the observed times/subgroups, nor when compared to the IN control group (Figure 3C).

ALT results for the subgroup INTR-NANO 90 days and the group TR-API 15 days were not included in the analyses because they were not available due to a technical equipment error at the time of analysis.

DISCUSSION

The elimination of Schistosomiasis mansoni as a public health problem has been the focus of disease control in the 21st century. As a solution, WHO (24) has proposed several actions to control transmission, including treatment with PZQ in endemic areas. This plan recommends treatment of schistosomiasis without prior diagnosis for active populations in endemic areas with transmission above 25% (26, 27). This strategy is known to provide treatment to people who are not only in the patent period of infection, but also in the prepatent period and without infection, which may or may not lead to drug resistance.

The present study verified that treatment with PZQ-API, which is similar to the formulation used for the treatment in endemic areas of schistosomiasis in Brazil, is efficient in the treatment of schistosomal infection in the pre-patent period
of infection, with significant reduction in EPG. These results validate the epidemiological strategy of the Brazilian Ministry of Health, and in line with the WHO, and refutes some previous work indicating that PZQ acts only during the patent period (28–33). These results become essential since early treatment could reduce morbidities associated with schistosomiasis, such as chronic anemia, liver fibrosis, portal hypertension, ascites, hepatic encephalopathy, and cognitive deficit (very common in children), among others (26, 34, 35). They may also lead to new strategies, such as treatment of school-age children, the primary victims of schistosomiasis (36–38) because they are more exposed to parasite transmission due to their leisure activities.

The present study also evaluated the PZQ-NANO therapy to offer an alternative for the treatment of *Schistosomiasis mansoni*. Nanoencapsulated pharmaceutical formulations represent an increasingly used drug delivery system, mainly for the treatment of some neglected diseases (39–45). Studies have pointed out that nanoformulations improve drug stability and bioavailability and direct it to the target zone, as well as optimize therapy and reduce adverse effects (41, 42). Both *in vivo* and *in vitro* studies (42–45) have demonstrated the effectiveness of nanoformulations containing PZQ at reducing the parasite burden of adult worms and improving liver damage in mice, while also damaging the integument of *S. mansoni* adults. Furthermore, encapsulation of PZQ reduces cytotoxicity, which indicates that this drug distribution system holds promise for the control of schistosomiasis (42–45). However, contrary to what can be observed in the literature, the nanoencapsulated formulation developed in Argentina and sent to Brazil for tests was not effective in treating schistosomal infection. This finding may be associated with the rigidity of the capsule of this formulation, which may have caused prolonged drug release in the gastrointestinal system, resulting in decreased concentrations absorbed by the intestines and, consequently, the action of the drug on adult worms.

The liver plays an essential role in schistosomal infection because it is where schistosomal cells mature to their adult form (46). Also, the induced formation of periovular granulomas in the liver by the immune response of the definitive host in response to the presence of worm eggs trapped in the walls of hepatic vessels and the development of periportal hepatic fibrosis characterize severe lesions of the organ that generate changes in liver enzymes (47, 48). For this reason, it is extremely important to evaluate liver function when determining the efficiency of PZQ at treating schistosomal infection. The liver enzymes evaluated in the present study were used to indicate possible lesions in hepatocytes and bile ducts of the liver.

According to the present results, the group of infected and untreated animals and the groups of infected animals treated with PZQ-API experienced higher levels of the enzyme ALP at 15 days p.i., after which it decreased until reaching its lowest levels at 60 days p.i. At 90 days p.i., serum ALP levels increased again, extensively, to surpass the reference range. ALP is routinely evaluated by hepatogram and is a parameter of great importance in diagnosing liver diseases. Some studies have related decreased serum ALP to an anemia characteristic of infection by *Schistosomiasis mansoni* (49, 50). Alternatively, increased ALP may be associated with changes in the intrahepatic bile duct, resulting from perportal fibrosis and/or periovular granuloma (51, 52). The results obtained here match the phases of *S. mansoni* in the definitive host. During the prepatent period, schistosomal cells are in the bloodstream feeding on red blood cells, while in the patent period, the adult worms are lodged in the hepatic and intestinal systems causing pathological changes in those organs.

The serum levels of TP were similar among the groups of infected animals (G. IN, G. INTR-API and G. INTR-NANO), showing lower results at 15 days p.i. and reaching the highest values at 90 days p.i. The increase in TP serum levels may be associated with a decompensated increase in globulins due to an aggravation of infection, which is observed mainly in animals with high parasite load (53).

The parameters ALT and AST were the most sensitive for pointing out differences in their plasma concentrations between the groups infected and treated with PZQ (INTR-API and INTR-NANO) and the most used indicators of hepatic lesions in the literature (50, 54). The animals treated with PZQ-API and PZQ-NANO at 15 days p.i. presented lower serum levels of ALT and AST than those infected and treated at the other times (30, 60 and 90 days p.i.), but similar to infected and untreated animals.

### TABLE 1 | Average amount of eggs per gram of feces (EPG) obtained from mice infected with *S. mansoni* in two periods: 60 days and 90 days after schistosomal infection.

| GROUPS          | 60 days p.i. |                    | 90 days p.i. |                    | EPG reduction (%) | P-value |
|-----------------|--------------|--------------------|--------------|--------------------|-------------------|---------|
|                 | N  | EPG (mean) | Intensity of infection | N  | EPG (mean) | Intensity of infection |     |
| IN              | 60 | 216.9<sup>a</sup> | Moderate | 50 | 92.4<sup>a</sup> | Light | 57.4 | 0.000<sup>*</sup> |
| INTR-API 15 DAYS | 10 | 78.8     | Light | 10 | 25.6     | Light | 67.5 | 0.005<sup>*</sup> |
| INTR-API 30 DAYS | 10 | 75.6     | Light | 10 | 27.6     | Light | 63.5 | 0.022<sup>*</sup> |
| INTR-API 60 DAYS | 10 | 130<sup>a</sup> | Moderate | 10 | 42.8     | Light | 67.0 | 0.014<sup>*</sup> |
| INTR-NANO 15 DAYS | 10 | 180.8    | Moderate | 10 | 210.4    | Moderate | −16.3<sup>b</sup> | 0.722 |
| INTR-NANO 30 DAYS | 10 | 218     | Moderate | 10 | 311     | Moderate | −42.6<sup>b</sup> | 0.250 |
| INTR-NANO 60 DAYS | 10 | 90.4<sup>b</sup> | Light | 10 | 224.4    | Moderate | −148.2<sup>b</sup> | 0.138 |
| Total           | 120 |          |                    | 110 |                |                  |       |

<sup>a</sup>EPG result for untreated infected animals. The P-value for the paired Mann-Whitney-Wilcoxon test. *Significant values for P < 0.05. *Negative values demonstrate an increase in EPG.
at 15 days p.i. (G. IN 15 days). For these same parameters, treatment of uninfected animals with PZQ-API or PZQ-NANO conferred lower serum levels than for infected animals. However, treatment at 30 days, 60 days and 90 days p.i. (PZQ-NANO and PZQ-API) caused a significant increase in ALT and AST when compared to the control group of infected and untreated animals.
(G. IN) and uninfected and treated animals (G. TR-API and G. TR-NANO). The increase in serum levels of these enzymes is related to necrosis of liver tissue caused by the formation of peri-ovular fibrosis and granulomas (55, 56). No references could be found in the literature that may explain the increase in serum levels of these enzymes after treatment. However, some
Synergistic effect may have occurred in liver metabolism due to *S. mansoni* infection and treatment with PZQ. It was also shown that treatment with PZQ-API in the prepatent period had less liver damage than treatment in the patent period. This can be explained by the biological cycle of the parasite. In the prepatent period (15 days), the adult worms of *S. mansoni* are not yet found in the liver and therefore have not mated or laid eggs. It should be noted that pathogenic liver lesions of schistosomiasis are associated with the presence of parasite eggs in the liver.

**CONCLUSIONS**

This study demonstrated that treatment with PZQ-API at 15 days and 30 days after schistosomal infection (pre-patent period) significantly reduces the number of eggs per gram of feces in infected mice. Serum levels of ALT, AST, and TP were lower in treated animals (with PZQ-API or PZQ-NANO) at 15 days p.i. than in animals treated at 30 days, 60 days, and 90 days p.i. Treatment with PZQ-NANO did not reduce EPG in infected animals in any period of the experiment. Therefore, more studies are needed to better understand the action mechanisms of PZQ on young forms of *S. mansoni* and develop more effective formulations with less side effects.

**DATA AVAILABILITY STATEMENT**

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

**ETHICS STATEMENT**

The animal study was reviewed and approved by Animal Ethics Commissions of Instituto Oswaldo Cruz.

**AUTHOR CONTRIBUTIONS**

CM and ÉP developed the study design. ÉP, CM, and MR developed all the experiments with the mice. CSi did all the biochemical analyses of the animals. CM and AM coordinated the project. AV and CSa developed the drugs and assisted with scientific support in this study. DR and DL were responsible for the formulation and characterization of the nanoencapsulated PZQ. All authors have reviewed the manuscript and contributed to the article and approved the submitted version.

**FUNDING**

This work was carried out with the support of the Coordination for the Improvement of Higher Education Personnel—Brazil (CAPES)—Financing Code 001 and with the support of the POM/PAEF of the Laboratory of Innovations in Therapies, Teaching, and Bioproducts (IOC/Fiocruz) and the Laboratory for Assessment and Promotion of Environmental Health (IOC/Fiocruz).

**ACKNOWLEDGMENTS**

We thank Daiani Cotrim de Paiva Campbell for all the assistance with animals in the study experiments, Dr. José Augusto Albuquerque dos Santos for technical support for handling the drugs used in the study, Vanessa Valladares, Jéssica Santos, and Valdir Almeida da Costa for assistance with Kato-Katz slide readings. We would also like to thank the Malacology Laboratory team (IOC/FIOCRUZ) for supplying the *S. mansoni* cercariae used in the study. Finally, we would like to thank the entire team of researchers and collaborators from the Laboratory of Experimental Pharmacotechnics (Farmanguinhos/FIOCRUZ), the Laboratory for Evaluation and Promotion of Environmental Health (IOC/Fiocruz), and the Laboratory of Innovations in Therapies, Teaching, and Bioproducts (IOC/Fiocruz) for all the ongoing support in this research.

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.848633/full#supplementary-material

**REFERENCES**

1. WHO. *Schistosomiasis*. (2020). Available online at: https://www.who.int/news-room/fact-sheets/detail/schistosomiasis (accessed November 2, 2021).
2. Bergquist NR, Leonardo LR, Mitchell GF. Vaccine-linked chemotherapy: can schistosomiasis control benefit from an integrated approach? *Trends Parasitol.* (2005) 21:112–7. doi: 10.1016/j.pt.2005.01.001
3. Colley DG, Buskindsuy AL, Secor WE, King CH. Human schistosomiasis. *Lancet.* (2014) 383:2253–64. doi: 10.1016/S0140-6736(13)61949-2
4. Noya O, Katz N, Pointier JP, Theron A and Noya BA. (2015). “Schistosomiasis in America”, in *Neglected Tropical Diseases—Latin America and the Caribbean*, eds. C. Franco-Paredes and J.I. Santos-Preciado (Vienna: Springer), pp. 12–38.
5. Ministério da Saúde (BR). *Portal da Saúde—Demográficas e socioeconômicas* [Internet]. (2021). Disponível em: http://tabnet.datasus.gov.br/cgi/deftohtm.exe?sih/cnv/nruf.def [Acessado em 31 de dezembro de 2021].

---

6. Olliaro P, Seiler J, Kuesel A, Horton J, Clark JN, Don R et al. Potential drug development candidates for human soil-transmitted helminthiases. *PLoS Negl Trop Dis.* (2011) 5:e1138. doi: 10.1371/journal.pntd.0001138
7. Katz N. “Terapêutica Clínica na Esquistossomose Mansoni” in: *Schistosoma mansoni e esquistossomose: uma visão multidisciplinar*, ed O.S. Carvalho (Rio de Janeiro, RJ: Editora FIOCRUZ), p. 849–70 (2008).
8. Xiao SH, Catto BA. Comparative in vitro and in vivo activity of racemic praziquantel and its levorotated isomer on *Schistosoma mansoni*. *J Infect Dis.* (1989) 159:589–92. doi: 10.1093/infdis/159.3.589
9. Doenhoff MJ, Sabah AA, Fletcher C, Webbe G, Bain J. Evidence for an immune-dependent action of praziquantel on *Schistosoma mansoni* in mice. *Trans R Soc Trop Med Hyg.* (1987) 81:947–51. doi: 10.1016/0035-9203(87)90360-9
10. Ciofi D, Pica-Mattoccia L. Praziquantel. *Parasitol Res.* (2003) 90:53–9. doi: 10.1007/s00436-002-0751-z
11. Botros S, Pica-Mattoccia L, William S, El-Lakkani N, Cioli D. Effect of praziquantel on the immature stages of Schistosoma haematobium. Int J Parasitol. (2005) 35:1435–7. doi: 10.1016/j.ijpara.2005.05.002
12. Barbosa MA, Pellegrino J, Coelho PMZ, Sampaio IBM. Quantitative aspects of the migration and evolutive asynchronism of Schistosoma mansoni in mice. Rev Inst Med Trop São Paulo. (1978) 20:121–32.
13. Faust EC, Jones CA, Hoffmann WA. Studies on schistosomiasis mansoni in Puerto Rico. III: Biological studies 2 The mammalian phase of the life cycle. P R Health Sci J. (1934) 10:133–96.
14. Vimeiro AC, Araújo N, Katz NKusel JR, Coelho PM. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models. Schistosomiasis: IN VIVO Models.
52. Leite LA, Pimenta Filho AA, Ferreira RD, Fonseca CS, Santos BS, Montenegro SM, et al. Splenectomy improves hemostatic and liver functions in hepatosplenic schistosomiasis mansoni. PLoS One. (2015) 10:e0135370. doi: 10.1371/journal.pone.0135370

53. Atta AM. Esquistossomose mansônica II — Evolução dos níveis de proteínas séricas e do perfil eletroforético por técnicas de immunoeletroforese quantitativa. Rev Saude Publ. (1981) 15:194–204. doi: 10.1590/S0034-89101981000200004

54. Elhenawy AA, Ashour RH, Nabih N, Shalaby NM, El-Karef AA, Abou-El-Wafa HS. Insulin growth factor inhibitor as a potential new anti-schistosoma drug: An in vivo experimental study. Biomed Pharmacother. (2017) 95:1346–58. doi: 10.1016/j.biopha.2017.09.015

55. Allam G. Immunomodulatory effects of curcumin treatment on murine schistosomiasis mansoni. Immunobiology. (2009) 214:712–27. doi: 10.1016/j.imbio.2008.11.017

56. Al-Olayan EM, El-Khadragy MF, Alajmi RA, Othman MS, Bauomy AA, Ibrahim SR, et al. Ceratonia siliqua pod extract ameliorates Schistosoma mansoni-induced liver fibrosis and oxidative stress. BMC Complement Altern Med. (2016) 16:434–44. doi: 10.1186/s12906-016-1389-1

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

**Publisher's Note:** All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

*Copyright © 2022 Paulino, Ribeiro de Lima, Viçosa, Silva, Salomon, Real, Leonardi, Mello Silva and Moraes Neto. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.*