Acta Tropica 218 (2021) 105909

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

In vitro and *in vivo* effects of P-MAPA immunomodulator on schistosomiasis

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ARTICLE INFO

Keywords:
Schistosoma mansoni
Schistosomiasis
P-MAPA
Praziquantel
Antischistosomal agents
Immunomodulators

ABSTRACT

Schistosomiasis is an infectious disease caused by helminth parasites of the genus *Schistosoma*; it is transmitted in over 78 countries. The main strategy for schistosomiasis control is treatment of infected people with praziquantel (PZQ). As PZQ-resistant strains have emerged, new anti-schistosomal agents have become necessary. We evaluated the *in vitro* and *in vivo* effect of P-MAPA, an aggregated polymer of protein magnesium ammonium phospholinoleate-palmitoleate anhydride with immunomodulatory properties; it is produced by *Aspergillus oryzae* fermentation. *In vitro*, P-MAPA (5, 50, and 100 μg/mL) damaged the *Schistosoma mansoni* tegument, causing thorn losses and tuber destruction in male worms and peeling and erosion in females after 24-h incubation. *In vivo*, P-MAPA (5 and 100 mg/kg, alone and combined with PZQ – 50 mg/kg) reduced the number of eggs by up to 69.20% in the liver and 88.08% in the intestine. Furthermore, granulomas were reduced up to 83.13%, and there was an increase in the number of dead eggs and a reduction of serum aspartate aminotransferase levels. These data suggest that P-MAPA activity can help improve schistosomiasis treatment and patients' quality of life.

1. Introduction

Schistosomiasis is an acute and chronic disease caused by helminth parasites of the genus *Schistosoma*. In 2015, an estimated 218 million people, more than half school-age children, were living in areas at high risk for the disease in over 78 countries (WHO, 2017). The disease is transmitted by *Schistosoma*-spp.-infected snails that release cercariae in watercourses. Cercariae penetrate the skin, and female worms initiate oviposition in the intestine around day 38 post-infection. By embolism, eggs reach mainly the liver and trigger host immunological reactions (Colley et al., 2014).

The current strategy for schistosomiasis control is the treatment of infected people with praziquantel (PZQ) (Katz and Coelho, 2008; McManus et al., 2018; WHO, 2017). However, PZQ has lost efficacy due to its long-term worldwide application, and PZQ-resistant and/or tolerant *Schistosoma mansoni* strains have been found (Danso-Appiah and De Vlas, 2002; Zwang and Olliaro, 2014). The situation urges the search for new anti-schistosomal agents to overcome PZQ resistance (Greenberg, 2014; Neves et al., 2015). One of the classes of drugs that appears to have potential in the treatment of schistosomiasis are immunomodulatory agents. Treatment with praziquantel associated immunomodulators in mice infected with *Schistosoma mansoni* appears to have promising potential (Silva et al., 2020).

P-MAPA, developed by the Farmabrasilis research network, is an aggregated polymer of protein magnesium ammonium phospholinoleate-palmitoleate anhydride with immunomodulatory properties. It is produced by *Aspergillus oryzae* fermentation. The compound increases the production of cytokines, especially interferon-gamma (IFN-γ) and interleukin 2 (IL-2), and stimulates the release of nitric oxide by macrophages (Farmabrasilis, 2008; Melo et al., 2014). P-MAPA has shown important activities against tumors (Fávaro et al., 2012), viruses (Durán et al., 2009), bacteria (Melo et al., 2001), and protozoa (Melo et al., 2014; Santiago et al., 2013). Toxicological studies determined that P-MAPA is safe in mice, since the LD50

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https://doi.org/10.1016/j.actatropica.2021.105909
Received 19 August 2020; Received in revised form 12 March 2021; Accepted 23 March 2021
Available online 28 March 2021
0001-706X/

Table 1
Experimental groups with the specific therapeutic description.

Groups	Number of animals
GI - *S. mansoni* infected and untreated	11
GII - *S. mansoni* infected and treated with P-MAPA 5 mg/kg	11
GIII - *S. mansoni* infected and treated with P-MAPA 5 mg/kg + PZQ 50 mg/kg	10
GIV - *S. mansoni* infected and treated with PZQ 50 mg/kg	10
GV - *S. mansoni* infected and treated with P-MAPA 100 mg/kg	9

intraperitoneally was obtained with a dosage of 2.71 ± 1.55 g/kg, which represents a dosage approximately 500 times higher than the therapeutic dosage used of the product, which is 5 mg/kg. (Farmabrasilis, 2008; Fávaro et al., 2012).

Here, its *in vitro* and *in vivo* action in a worm, *S. mansoni*, and its potential as an adjuvant therapy in the treatment of schistosomiasis were evaluated for the first time.

2. Materials and methods

2.1. Study design

S.-mansoni-infected male mice were treated or untreated with P-MAPA and PZQ in different treatment regimens. One-hundred-eleven Swiss male mice (60 for the *in vitro* and 51 for the *in vivo* studies) at 28–30 g and 28 days of age were provided by the Aggeu Magalhães Institute (IAM) breeding stock. Before the experiments, the animals were submitted to quarantine to reduce stress effects. The animals were identified by coding to minimize the risk of bias by the observers. They were housed in boxes on shelves under constant temperature (21 ± 2 ºC) and brightness (12 h light/12 h dark) and had access to food and water *ad libitum*. The study protocol (97/2016) was approved by the IAM Ethics Committee on Animal Studies.

2.2. Infection of mice

The mice were individually infected with 80 cercariae from *S. mansoni* LE strain (Luiz Evangelista) kept in *Biomphalaria glabrata* snails raised in the Laboratory of Malacology of IAM/FIOCRUZ. The infection was performed percutaneously, with exposure for 1 h under artificial light.

2.3. Experimental groups and drugs

Mice were randomly allocated into five experimental groups (GI to GV), according to the therapeutic intervention (Table 1).

P-MAPA holds the Orphan Drug Designation (https://www.accessdata.fda.gov/scripts/opdlisting/oopd/detailedIndex.cfm?cfgridkey=721319) status by the U.S. Food and Drug Administration (FDA), was developed and kindly provided by Farmabrasilis (Valinhos, SP, Brazil), a non-profit research network. Praziquantel was purchased from Sigma (Sigma Aldrich, St. Louis, MO, USA). Mice were treated with a single 50 mg/kg oral dose of PZQ and/or intraperitoneal P-MAPA injection. Both drugs were diluted in 5% Pluronic F-127 (Sigma Aldrich, St. Louis, MO, USA). P-MAPA was given at 5 mg/kg/day (suggested therapeutic dose) for 8 days (days 60–67 post-infection) and at 100 mg/kg at day 60, 64 and 67 post-infection to observe the effects of the drug during the course of infection.

2.4. In vitro test in adult worms

Two pairs of adult worms, recovered from infected mice, were placed in each well of a 24-well culture dish that contained RPMI 1640 medium supplemented with antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin) and 10% fetal bovine serum (FBS). The plates were incubated at 37 °C and 5% CO_2 for 2 h.

A stock solution of P-MAPA was prepared and added to the wells at concentrations of 5, 50, or 100 µg/mL. PZQ (positive control) was used at a concentration of 0.5 µg/mL. The negative control contained only supplemented RPMI media. Each dose was tested in triplicate; the final volume in each well was 2 mL.

The parasites were monitored for survival over 24 h on an inverted microscope. Phenotypic changes were scored based on a motility scale that ranged from 0 to 3 (Ramirez et al., 2007): 3, active worms with normal body movements; 2, slow worms with delayed body movements or only movements at the extremities of the anterior and posterior regions; 1, very slow worms, with occasional movement of the extremities or intestine; 0, dead worms, with total absence of movement. The experiments were performed in triplicate and repeated.

2.5. Tegument evaluation of S. mansoni by scanning electron microscopy

Adult worm pairs were treated with 5, 50, or 100 µg/mL P-MAPA, RPMI 1640 (negative control), or 0.5 µg/mL PZQ (positive control) for 24 h. Subsequently, they were fixed in Karnovsky's fixative and stored under refrigeration.

Worms were washed three times with a buffer solution (sodium cacodylate, pH = 7.2) and fixed in 2.5% glutaraldehyde (pH = 7.4) for 24 h and later in 1% osmium tetroxide at room temperature for 1 h. All worms were washed several times in 100% ethanol to be dehydrated, dried in liquid CO_2, stacked, gold plated, and scanned in a JEOL JSM-5600 electron microscope (Jeol Ltd., Tokyo, Japan) at Keiso Asami Immunopathology Laboratory (LIKA), UFPE. All measurements were performed in µm.

2.6. In vivo tests

2.6.1. Worm recovery

Animals were anesthetized with 115 mg/kg ketamine hydrochloride and 10 mg/kg xylazine hydrochloride. Worms were recovered from the hepatic portal system by an infusion technique (Alencar et al., 2016). The worms from each mouse were sedimented for approximately 20 min in Petri dish with RPMI 1640 medium to allow identification, examination, sex determination, and counting.

2.6.2. Egg counting

The number of eggs/g liver was counted after 4% KOH digestion, according to the method described by Cheever and Anderson (Cheever and Anderson, 1971). On the oogram, the small intestine of mice was cut into approximately 1 cm segments, compressed between slides, and examined using an optical microscope. *S. mansoni* eggs (mature, immature and dead) were counted in three segments for each mouse to calculate the average number of eggs. This classification was done following the method specifications under the microscope visualization: mature – embryo was occupying its entire internal area; immature – embryo was not visible or did not occupy the internal area of the egg; dead – embryo or miracide was retracted (Pellegrino et al., 1962).

2.6.3. Histopathology

The central portion of the right liver lobe was removed from all animals and fixed in 10% formalin. The fragments were dehydrated in increasing concentrations of ethanol, diaphanized in xylol, and embedded in paraffin. Five µm sections were stained with hematoxylin-eosin (HE). All granulomas found in three random fields of the histological section were quantified. The images were captured with a Labomed Lx400 microscope (Labomed Inc. Los Angeles, CA, US) attached to a 1.3 MP USB 2.0 Moticam 1000 digital camera and using Motic Images Plus 2.0 software (Motic Incorporation Ltd., Hong Kong).

2.6.4. Biochemistry

On day 68 post-infection, blood was collected under anesthesia from

Table 2
Worm motility score after 24 h incubation with praziquantel (PZQ) or P-MAPA.

GroupsMotility score	Worms percentage (%)			
	0	1	2	3
Control				100
PZQ 0,5 μg/ml	100			
P-MAPA 5 μg/ml		25	75	
P-MAPA 50 μg/ml		100		
P-MAPA 100 μg/ml		100		

the vena cava. Serum was obtained after centrifugation at 1000x*g* for 15 min. The serum levels of the enzymes aspartate transaminase (AST) and alanine aminotransferase (ALT) were evaluated using the BioSystem® kit (Barcelona, Spain).

2.6.5. Statistical analysis

Data are expressed as mean ± standard deviation (SD). Analysis of variance (ANOVA) was used for comparison among groups. When ANOVA revealed a significant difference, Bonferroni's post-test was used to identify the difference between groups. Differences were considered significant when $p < 0.05$. GraphPad Prism 5.01 (GraphPad Software, San Diego, CA, US) was used for statistical analysis.

3. Results

3.1. In vitro effect of P-MAPA on S. mansoni adult worms

P-MAPA at 5, 50, or 100 μg/mL reduced the motility of adult worms. Table 2 details the schistosomicidal effect of P-MAPA on *S. mansoni* adult worm motility after 24-h incubation. There was no adhesion of suction cups or egg laying. Fig. 1A-E shows the preserved morphology of the negative control adult worms and Fig. 1F-I the worm characteristics after *in vitro* PZQ treatment. Fig. 2A-B shows female and male worm treated with 5 μg/mL P-MAPA for 24 h and indicates peeling and erosion of the tegument, showing areas with cracks.

The effects of 50 μg/mL P-MAPA on female and male worm are shown in the Fig. 2C-D. There were peeling and erosion of the tegument, exposure of the submuscular tissue, areas with cracks and presence of holes. At the same concentration in the male worm, there was a total destruction of the tubers with exposure of submuscular tissue and damaged sensorial structures (Fig. 2C-D). Fig. 2E depicts swelling on the tegument and erosion in the female's body as a result of *in vitro* 100 μg/mL P-MAPA exposure. Loss of spines, destruction of tubers, bubbles and presence of holes were observed at the same concentration on the male worm (Fig. 2F).

3.2. Worm recovery

As shown in Fig. 3, all tested treatments significantly reduced the total number of worms recovered by perfusion when compared to the untreated infected mice.

3.3. Effect of P-MAPA on the number of eggs/g liver or intestine

Combined 5 mg/kg P-MAPA + 50 mg/kg PZQ or 100 mg/kg P-MAPA alone significantly decreased the number of eggs/g of liver compared to the untreated group. The reductions were 67.31% and 69.20%, respectively. All treatment regimens significantly reduced the number of eggs/g of intestine compared to untreated infected mice. Combined 5 mg/kg P-MAPA + 50 mg/kg PZQ presented the greatest egg count reduction in the intestine (88.08%) as well as the liver (Table 3).

The oviposition pattern was significantly reduced in mature eggs in all treatment groups when compared to the untreated infected group (Table 4). Only the 5 mg/kg P-MAPA treatment regimen did not significantly reduced immature eggs in comparison to the infected control group. Combined 5 mg/kg P-MAPA + 50 mg/kg PZQ or 50 mg/kg PZQ alone also reduced the number of immature eggs when compared with the 5 mg/kg-P-MAPA-treated group and untreated infected mice.

The 5 mg/kg P-MAPA and 50 mg/kg PZQ treatments significantly increased the counts of dead eggs when compared with the control group. Furthermore, combined 5 mg/kg P-MAPA + 50 mg/kg PZQ or 100 mg/kg P-MAPA alone also significantly increased the number of dead eggs when compared with 5 mg/kg-P-MAPA-treated mice. These results showed that P-MAPA seems to modulate the oviposition pattern of *S. mansoni*.

3.4. Effect of P-MAPA on liver granulomas

Table 5 shows the significant reduction in the number of granulomas for all studied treatment regimens when compared to the untreated infected group. Beyond presenting differences regarding the infected mice, 100 mg/kg P-MAPA also significantly reduced the granuloma number when compared to 50 mg/kg-PZQ-treated mice.

Fig. 4 depicts hepatic granulomas in the different treatment regimens. The hepatic tissue of a control animal, without infection and without treatment, is presented in Fig. 4A, and the infected and untreated group is shown in Fig. 4B. Histological sections with hepatic granulomas treated with 50 mg/kg PZQ or 5 mg/kg P-MAPA are shown in Fig. 4C and 4D, respectively. Hepatic granulomas under the effect of 5 mg/kg P-MAPA treatment + 50 mg/kg PZQ or 100 mg/kg P-MAPA are shown in Fig. 4E and 4F, respectively.

3.5. Liver enzyme levels

The serum levels of AST and ALT in the treatment groups are shown in Fig. 5. There was an apparent reduction in ALT levels in all treatment regimens compared to the infected and untreated group, but the reduction was not statistically significant. AST was reduced in all treatment groups, but the difference was only statistically significant in the 100 mg/kg-P-MAPA-treated mice.

4. Discussion

As a strategic target for schistosomicidal drugs, the *S. mansoni* tegument plays a key role in nutrition, excretion, signal transduction, evasion, osmoregulation, immune modulation, and parasite-host interactions (Jones et al., 2004; Mulvenna et al., 2010). Indeed, tegumentary changes - vacuolations, peeling of the tegument, tubercles and spines, increased membrane permeability and Ca^{2+} influx, absence of phosphorylation in α1- and β-calcium channels, membrane depolarization in adult worms, schistosomulae, a contraction and muscular paralysis that act on parasite neurotransmitters - are observed after exposure of *S. mansoni* to low PZQ concentrations (Lorsuwannarat et al., 2013; Xiao et al., 2007). As young worms are less susceptible to PZQ than adults (Colley et al., 2014; Araújo et al., 2020a), and decreased susceptibility to PZQ is reported (Melman et al., 2009; Zwang and Olliaro, 2014), the association of drugs for the treatment of schistosomiasis has been emphasized to increase therapeutic efficiency and deter the emergence of resistant strains (Katz and Coelho, 2008).

At all concentrations evaluated *in vitro*, P-MAPA presented powerful activity against adult male and female worms and severely damaged the worm tegument. These damages included the loss of spicules, tuber destruction, depression and formation of bubbles in male worms; and peeling, erosion, and swelling in females. Consistently, other *in vitro* studies on natural products reported that *S. mansoni* male worms are often more susceptible to treatments compared to female worms (Melo et al., 2011; Sanderson et al., 2002; Araújo et al., 2019; 2020b).

Beyond damaging worm tegument, decreasing worm motility, wrinkling worms, and halting oviposition for 24 h, P-MAPA reduced the recovery of worms by up to 50% by the portal hepatic system compared

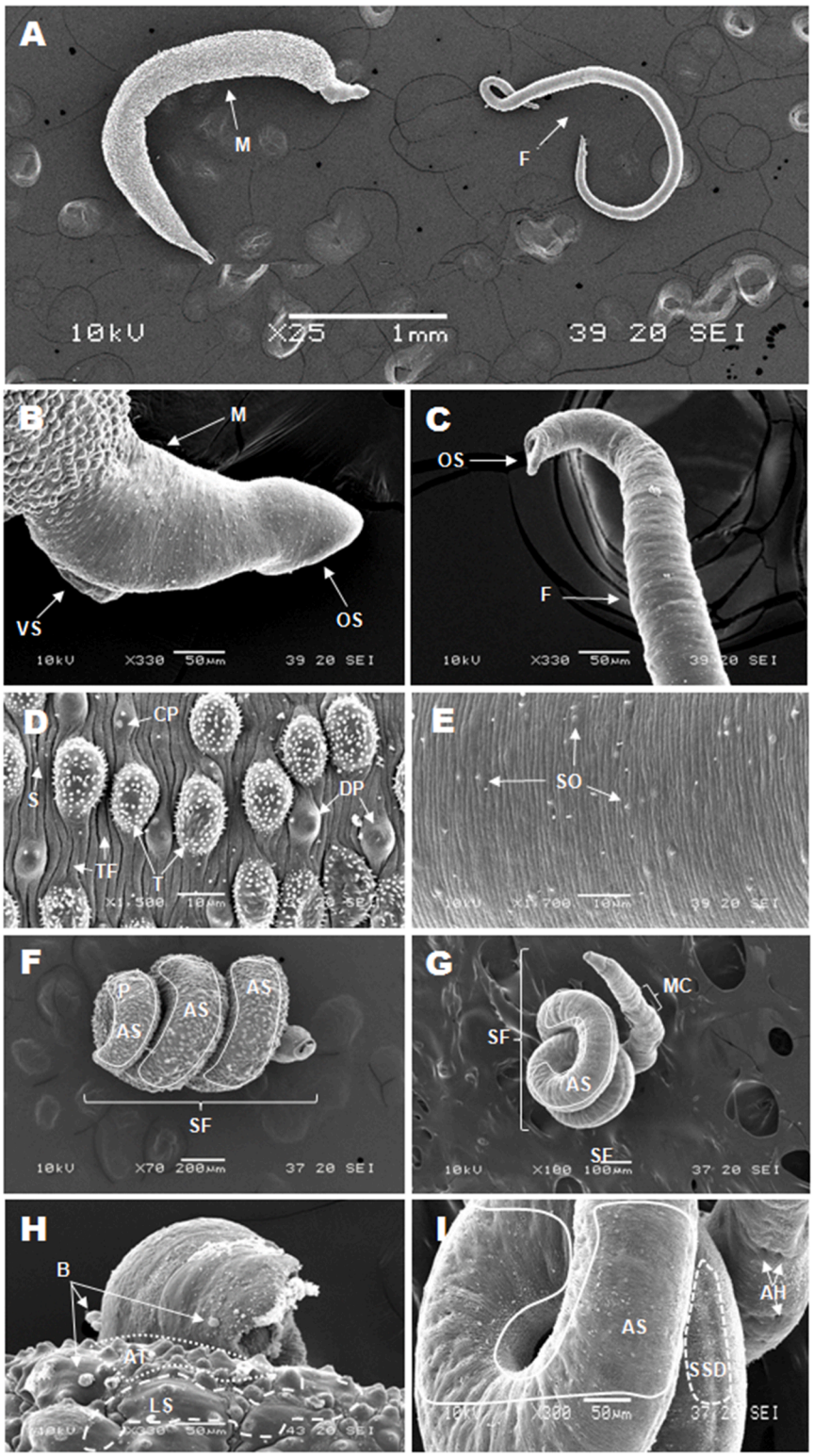

Fig. 1. (A-I) Electromicrographs of adult *S. mansoni* from the negative control group (A-E: RPMI 1640 medium) and positive control group (F-I: PZQ). (A, 25x) *S. mansoni* couples (*M*=male and *F*=female). On the anterior region of male (B, 330x) and female (C, 330x) worms ventral sucker (VS) and oral sucker (OS). (D, 1,500x) On the enlarged view of dorsal male region, it is possible to observe tubercles (T) with spines, parallel folds between tubercles (TF), spines (S), ciliated papillae (CP), and dome-shaped papillae (DP). (E, 1,700x) Female with longitudinal lines and well-distributed sensory organs (SO). (F, 70x and G, 100x) Male and female in spiral format (SF) respectively, both with extensive swelling area (AS). Still being observed peeling (P) in the male and muscle contraction (MC) at the female. (H, 330x) On male median and anterior regions, the presence of bubbles (B) emerging around the tubercles of the male worm. On the dorsal region of male worms we observed agglomerated tubercles (AT) or juxtaposed and loss of spicules (LS). (I, 330x) Tegument with extensive areas of swelling (AS), and highlighted damaged sensorial structures (SSD) and appearance of holes (AH).

to the untreated infected group. Additionally, combined P-MAPA + PZQ or 100 µg/mL P-MAPA alone reduced the number of eggs/g of liver by 67.31 and 69.20%, respectively, compared to the infected mice without treatment.

Schistosoma spp. couples that remain in the vertebrate host blood system exhibit high oviposition rates. This activity results in immunopathological lesions characterized by inflammation and fibrosis in the target organ (Santos et al., 2018; McManus et al., 2018; Schwartz and Fallon, 2018). The most important pathogenic event in schistosomiasis is the formation of hepatic granuloma and peri-portal hepatic fibrosis (Santos et al., 2018). Eggs are initially laid by females in the mesenteric vessels and subsequently carried by the bloodstream to other organs, including the liver. Human and experimental schistosomiasis is directly related to the development of granulomatous reactions due to the release of soluble antigens produced by *S. mansoni* eggs, which induce an immune response in the host (Hams et al., 2013; Schwartz and Fallon, 2018).

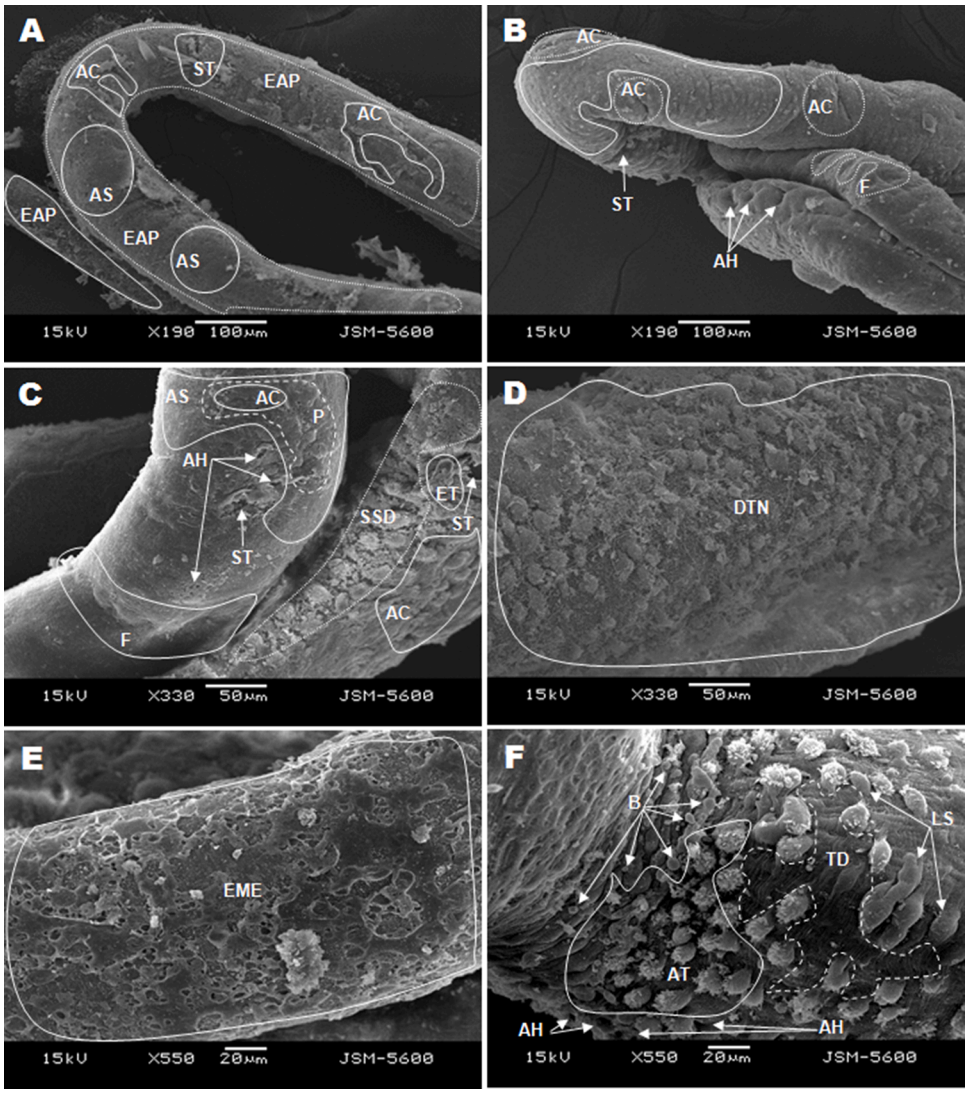

Fig. 2. (A-F). Electromicrographs of *S. mansoni* adult couples exposed by 24 h to 5, 50 e 100 µg/mL P-MAPA. 5 µg/mL P-MAPA (A, 190x) shows extensive area of peeling (EAP) of the female worm, with areas of swelling (AS) and cracks (AC) in addition to exposure of the submuscular tissue (ST). In (B, 190x) areas with cracks (AC) and evidence of submuscular tissue (ST) in female and evidence of furrows (F) and appearance of holes (AH) in male worm. At 50 µg/mL P-MAPA (C, 330x) shows peeling (P), areas with cracks (AC), swelling (AS), furrows (F) and presence of holes (AH) with different levels of severity, some with exposure of subtegumental tissue (ST) in female worm. While in the male worm it highlights damaged sensorial structures (SSD), eruption of tubercle (ET), exposure of submuscular tissue (ST) and area with cracks (AC). In the median region of the male worm (D, 330x) destruction of numerous tubers (DTN). At 100 µg/mL P-MAPA, the female worm (E, 550x) presented several erosions with exposure of the musculature (EME) and on the male worm (F, 550x), we observed tubercles displacement (TD), agglomerated tubercles (AT), presence of bubbles (B), loss spicules (LS) and appearance of holes (AH).

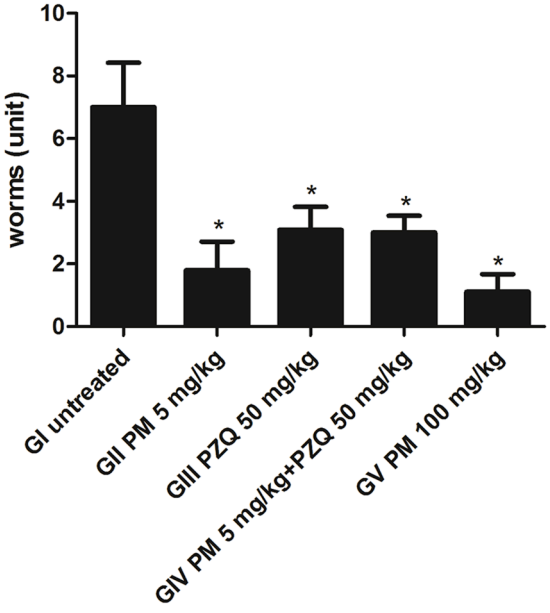

Fig. 3. *Schistosoma mansoni* worm recovery after treatment schemes. *Significant difference compared to untreated infected group (p <0.05).

Mature eggs play a key role in the pathological aspects of schistosomiasis and in the disease transmission. Thus, the high ovicidal effect observed in the oogram for all P-MAPA treatment regimens can surely contribute to decrease the disease transmission, especially at unhealthy environments. The reduction in the number of eggs may be related to the fecundity of the worms. Consistently, P-MAPA acted on the male and female worm tegument and precluded egg laying by the females, actions that impact the worm's oviposition pattern.

Table 3
Liver and gut egg count after isolated treatment with P-MAPA or in association with praziquantel (PZQ) in mice infected with *Schistosoma mansoni*.

Groups	Liver egg count/g × 10^3	% Reduction	Gut egg count/g × 10^3	% Reduction
S. mansoni infected and untreated	16,49 ± 3,40	–	10,79 ± 3,09	–
P -MAPA 5 mg/kg	8,84 ± 1,24	46,38%	1,74 ± 0,41[a]	83,86%
P-MAPA 5 mg/kg + PZQ 50 mg/kg	5,39 ± 1,18[a]	67,31%	1,28 ± 0,28[a]	88,08%
PZQ 50 mg/kg	8,41 ± 1,80	48,94%	2,14 ± 0,33[a]	80,13%
P-MAPA 100 mg/kg	5,07 ± 3,04[a]	69,20%	2,29 ± 1,36[a]	78,76%

Values are expressed as average ± SD.
[a] significant difference when compared with ***S. mansoni* infected and untreated** (p<0,05).

Table 4
Oogram pattern and egg development in the gut after isolated P-MAPA treatment or in association with praziquantel (PZQ) in *S. mansoni*-infected mice.

Groups	%Mature eggs	%Immature eggs	%Dead eggs
S. mansoni infected and untreated	63,55 ± 29,31	32,05 ± 16,26	4,40 ± 1,96
P-MAPA 5 mg/kg	33,37 ± 16,13[a]	29,16 ± 15,65	37,47 ± 21,35[a]
P-MAPA 5 mg/kg + PZQ 50 mg/kg	43,51 ± 9,34[a]	15,69 ± 3,18 [a,b]	40,79 ± 12,87[b]
PZQ 50 mg/kg	34,89 ± 15,36[a]	16,55 ± 4,64[a,b]	48,55 ± 17,43[a]
P-MAPA 100 mg/kg	43,49 ± 25,00[a]	30,50 ± 15,46[a]	25,98 ± 21,24[b]

Values are expressed as average ± SD.
[a] significant difference when compared with ***S. mansoni* infected and untreated** ($p<0,0001$).
[b] significant difference when compared with P-MAPA 5 mg/kg (p<0,0001).

Table 5
Granuloma amount after isolated P-MAPA treatment or in association with praziquantel (PZQ) in *S. mansoni*-infected mice.

Groups	Granuloma count/3 fields/ 40x	% Reduction
S. mansoni infected and untreated	27,45 ± 4,14	
P-MAPA 5 mg/kg	10,00 ± 1,51[a]	63,57%
P-MAPA 5 mg/kg + PZQ 50 mg/kg	8,18 ± 1,66 [a]	70,20%
PZQ 50 mg/kg	16,82 ± 1,71[a]	38,72%
P-MAPA 100 mg/kg	4,63 ± 2,17 [a,b]	83,13%

Values are expressed as average ± SD.
[a] significant difference when compared with ***S. mansoni* infected and untreated** ($p<0,004$).
[b] significant difference when compared with PZQ 50 mg/kg (p<0,004).

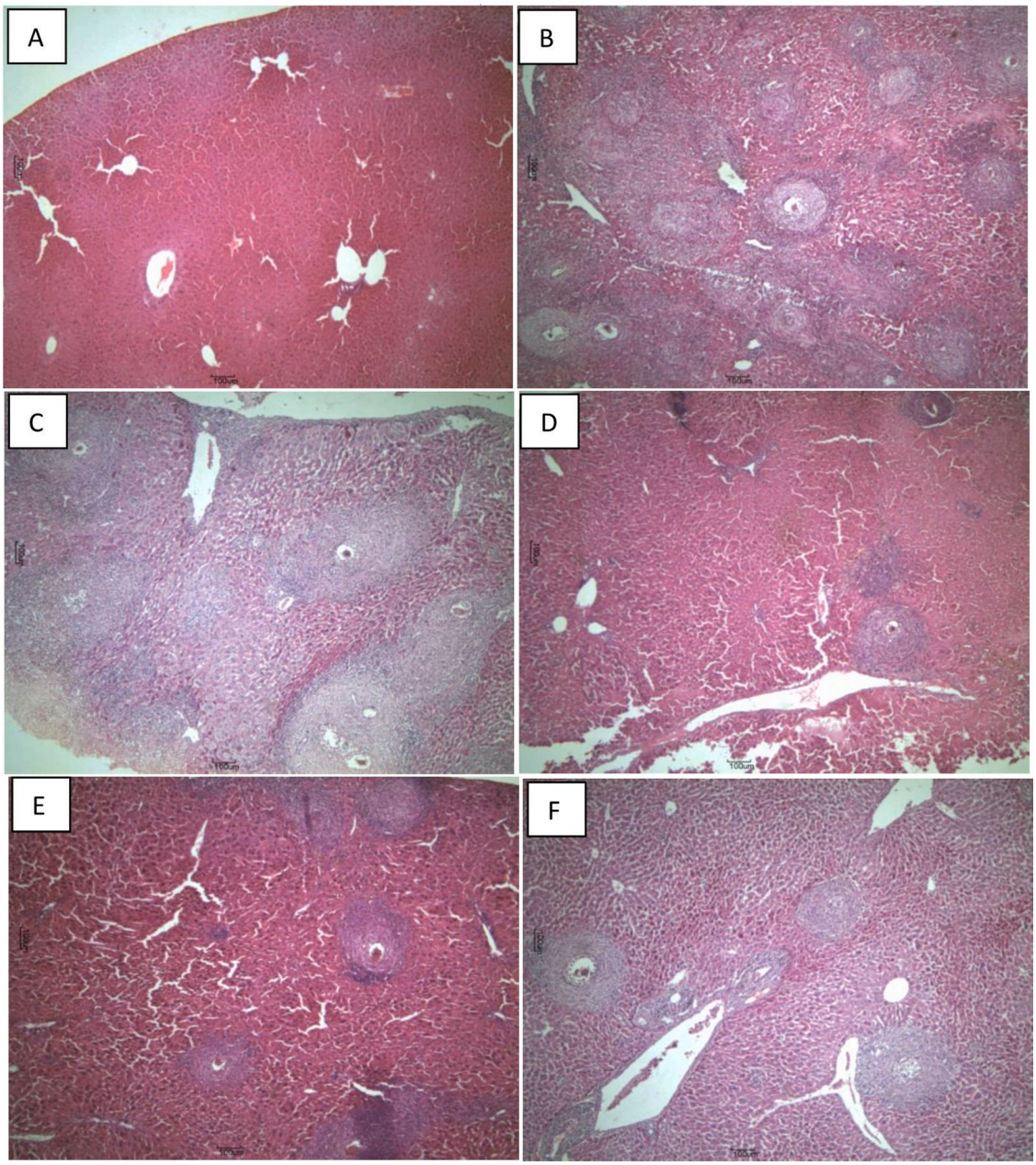

Fig. 4. (A-F). Hepatic granuloma count in the different treatment groups. Images are for hematoxylin-and-eosin stained sections observed with a 40X objective lens. Images are for: control liver without infection (A); infected control without treatment (B); treatment with 50 mg/kg PZQ (C); treatment with 5 mg/kg P-MAPA (D); treatment with 5 mg/kg P-MAPA + 50 mg/kg PZQ (E); treatment with 100 mg/kg P-MAPA (F).

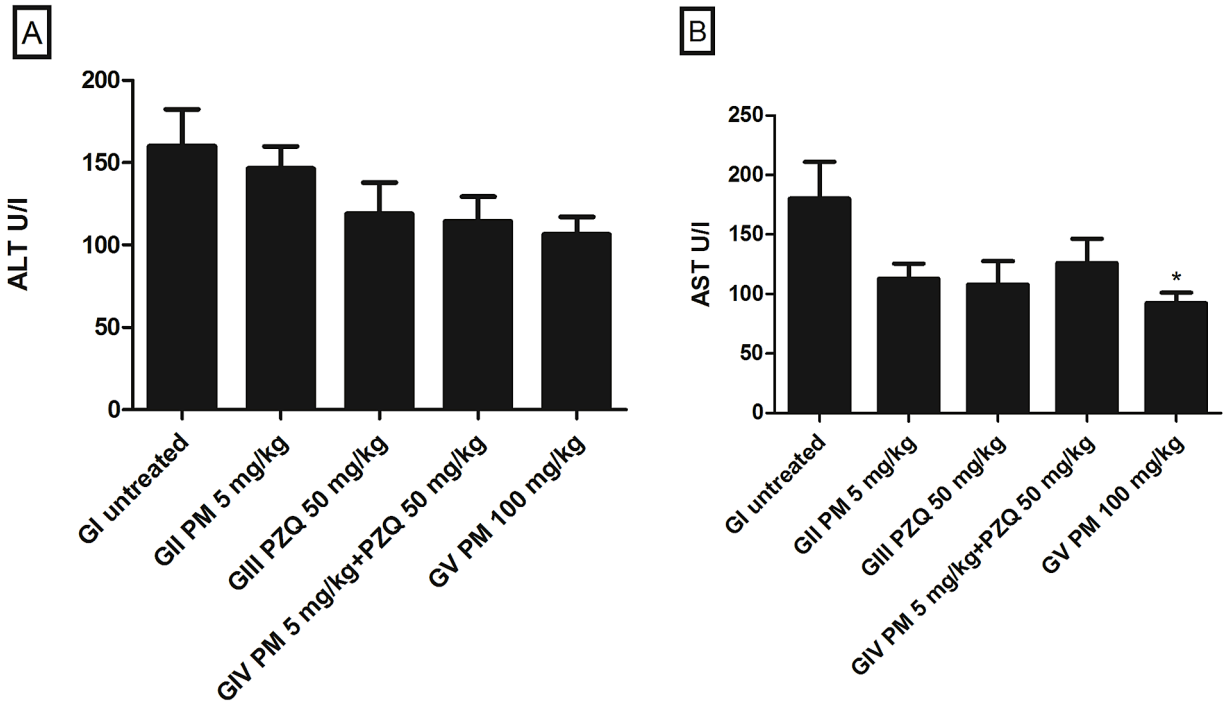

Fig. 5. Effect of different treatment schemes on liver function enzyme activity. Data are shown for alanine amino transferase (ALT; A) and aspartate aminotransferase (AST; B). *Significant difference compared to the untreated infected group ($p < 0.05$).

In all studied treatment regimens, the number of hepatic granulomas was significantly reduced, along with the number of eggs/g of liver; this reduction ranged from 46.38% to 69.20%. Histopathological changes in liver cells are often related to ALT elevation (Mahmoud et al., 2002). The changes can be easily observed in experimental models, but in humans they only occur during late stages of the disease and may disappear if the treatment works adequately. Serum AST and ALT levels may protect the liver from schistosomiasis, consistent with similar results from the use of *Zingbar officinale* extract with immunomodulatory potential (Aly and Mantawy, 2013). Although ALT levels were apparently reduced in infected mice treated with P-MAPA, PZQ, or combination of the two, there was no statistically significant difference when compared to circulating levels in untreated infected animals. On the other hand, AST levels were apparently reduced in infected and treated mice, although this difference was only statistically significant for treatment with 100 mg/kg P-MAPA when compared to circulating levels of the infected and untreated animals.

P-MAPA mechanisms of action are not fully elucidated. The direct effects of P-MAPA on the worm tegument were unexpected, but we hypothesize that parts of the compound, which contains ions, proteins and fatty acids, provoke tegument modification. P-MAPA also exerts immunomodulatory effects, activating Toll-like Receptors (TLRs) and the interferon (IFN) signaling pathways (Fávaro et al., 2012) and associated host immune cells. We believe that these immune pathways activation contribute to the schistosomiasis burden attenuation observed in the current study (reduced egg oviposition, worm recovery, as well as hepatic granulomas). P-MAPA seems to change the schistosomiasis-induced immune profile and oviposition pattern, improving disease control.

In translational terms, the conversion of animal dose to human dose is performed by normalizing their body surface area (Reagan-Shaw et al., 2008). Thus, a daily dose of 100 mg/kg P-MAPA used in the mouse experiments is equivalent to a human dose of 8.1 mg/kg/day, and a daily dose of 5 mg/kg is equivalent to a human dose of 0.40 mg/kg/day. Crucially, the 5 mg/kg treatment regimen used in mice and its human equivalent is smaller than the P-MAPA doses used in a phase I clinical trial. No adverse events were reported in these trials, data that emphasize its potential for the treatment of schistosomiasis (Farmabrasilis, 2008).

P-MAPA exerted considerable activity against *S. mansoni* *in vitro* and *in vivo* tests. The experimental data demonstrated that P-MAPA substantially affected the motility of adult worms and the tegument of males and females *in vitro*. P-MAPA monotherapy, or in combination with PZQ, reduced: the number of worms recovered, the number of eggs (up to 69.20% in the liver and up to 88.08% in the intestine), the number of hepatic granulomas (up to 83.13%), and serum AST levels in infected mice. On the other hand, few pieces of the P-MAPA activity on schistosomiasis still need to be evaluated, such as the drug specific mechanism of action on the *S. mansoni* structures and infected host, its effect on young worms and its consequences in the oviposition or sexual organs. Even so, the described data, associated with the lack of side effects, support that P-MAPA can be of great importance to improve the treatment of schistosomiasis and quality of life of the people affected by this neglected disease.

Author contributions

J. C. S. Silva, I. S. Nunes, F. L. Melo and B. M. Carvalho designed the study protocol. J. C. S. Silva, C. R. B. Lins, S. S. Lacerda, R. E. M. Ramos, H. D. A. Araújo, M. R. Melo-Junior, L. C. Alves, F. A. Brayner, I. S. Nunes, F. L. Melo, and B. M. Carvalho carried out the assays and were involved in the analysis and interpretation of all data. J. C. S. Silva, H. D. A. Araújo, I. S. Nunes, F. L. Melo and B. M. Carvalho contributed to drafting the manuscript and/or critically revising the paper and intellectual content. All authors read and approved the final manuscript.

Declaration of Competing Interest

We wish to confirm that there are no known conflicts of interest associated with this publication.

Acknowledgments

The authors thank Silvania Tavares Paz from the Histotechnology

laboratory from the Pathology Postgraduate Program at UFPE. This work was supported by the Higher Education Personnel Improvement Coordination - Brazil (CAPES) - Finance Code 001. We also thank the National Council for Scientific and Technological Development (CNPq - grant Universal 475751/2013-2) and the Pernambuco State Science and Technology Support Foundation (FACEPE - grant APQ-0428-2.07/14). H.D.A. Araújo thanks FACEPE for the Researcher Fixation Scholarship (BFP-0080–2.08/20).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.actatropica.2021.105909.

References

Alencar, A.C., Santos, T.a.S., Neves, R.H., Lopes Torres, E.J., Nogueira-Neto, J.F., Machado-Silva, J.R., 2016. Simvastatin and artesunate impact the structural organization of adult *Schistosoma mansoni* in hypercholesterolemic mice. Exp. Parasitol. 167, 115–123. https://doi.org/10.1016/j.exppara.2016.05.007.

Aly, H.F., Mantawy, M.M., 2013. Efficiency of ginger (*Zingbar officinale*) against *Schistosoma mansoni* infection during host-parasite association. Parasitol. Int. 62, 380–389. https://doi.org/10.1016/j.parint.2013.04.002.

Araújo, H.D.A., Aires, A.L., Soares, C.L.R., Brito, T.G.S., Nascimento, W.M., Martins, M.C. B., Silva, T.G., Brayner, F.A., Alves, L.C., Silva, N.H., Albuquerque, M.C.P.A., Lima, V.L.M, 2019. Usnic acid potassium salt from *Cladonia substellata* (Lichen): synthesis, cytotoxicity and *in vitro* anthelmintic activity and ultrastructural analysis against adult worms of *Schistosoma mansoni*. Acta Trop 192, 1–10. https://doi.org/10.1016/j.actatropica.2018.12.024.

Araújo, H.D.A., Santos, V.H.B., Brayner, F.A., Alves, L.C., Silva, N.H., Albuquerque, M.C. P.A., Aires, A.L., Lima, V.L.M., 2020a. *In vitro* activity of usnic acid potassium salt against different developmental stages of *Schistosoma mansoni*: an ultrastructural study. Acta Trop 201, 105159. https://doi.org/10.1016/j.actatropica.2019.105159.

Araújo, H.D.A., Silva, N.H., Albuquerque, M.C.P.A., Aires, A.L., Lima, V.L.M., 2020b. Potassium usnate, a water-soluble usnic acid salt, shows enhanced activity against *Schistosoma mansoni in vitro*. Exp. Parasitol. 208, 107779 https://doi.org/10.1016/j.exppara.2019.107779.

Cheever, A.W., Anderson, L.A., 1971. Rate of destruction of *Schistosoma mansoni* eggs in the tissues of mice. Am. J. Trop. Med. Hyg. 20, 62–68. https://doi.org/10.4269/ajtmh.1971.20.62.

Colley, D.G., Bustinduy, A.L., Secor, W.E., King, C.H., 2014. Human schistosomiasis. Lancet 383, 2253–2264. https://doi.org/10.1016/S0140-6736(13)61949-2.

Danso-Appiah, A., De Vlas, S.J., 2002. Interpreting low praziquantel cure rates of *Schistosoma mansoni* infections in Senegal. Trends Parasitol 18, 125–129. https://doi.org/10.1016/s1471-4922(01)02209-7.

Melo, A., Justo, G.Z., Queiroz, M.L.S., 2001. Stimulation of myelopoiesis in Listeria monocytogenes-infected mice by an aggregated polymer isolated from *Aspergillus oryzae*. Hum. Exp. Toxicol. 20, 38–45. https://doi.org/10.1191/096032701669333804.

Melo, N.I., Magalhaes, L.G., Carvalho, C.E., Wakabayashi, K.A., Aguiar, G.P., Ramos, R. C., Mantovani, A.L., Turatti, I.C., Rodrigues, V., Groppo, M., Cunha, W.R., Veneziani, R.C., Crotti, A.E., 2011. Schistosomicidal activity of the essential oil of *Ageratum conyzoides* L. (Asteraceae) against adult *Schistosoma mansoni* worms. Molecules 16, 762–773. https://doi.org/10.3390/molecules16010762.

Durán, N., Gowen, B.B., Costa, F.T., Justo, G.Z., Brocchi, M., Nunes, O.S., Nunes, I.S., 2009. A biotechnological product and its potential as a new immunomodulator for treatment of animal phlebovirus infection: punta Toro virus. Antiviral. Res. 83, 143–147. https://doi.org/10.1016/j.antiviral.2009.04.006.

Farmabrasilis, 2008. P-MAPA immunomodulator. http://www.farmabrasilis.org.br/todos_conteudos_interna.php?idioma=eng&id=110. (Accessed 20 april 2020).

Fávaro, W.J., Nunes, O.S., Seiva, F.R., Nunes, I.S., Woolhiser, L.K., Durán, N., Lenaerts, A.J., 2012. Effects of P-MAPA immunomodulator on toll-like receptors and p53: potential therapeutic strategies for infectious diseases and cancer. Infect. Agent. Cancer. 7, 14. https://doi.org/10.1186/1750-9378-7-14.

Greenberg, R.M., 2014. Schistosome ABC multidrug transporters: from pharmacology to physiology. Int. J. Parasitol. Drugs. Drug. Resist. 4, 301–309. https://doi.org/10.1016/j.ijpddr.2014.09.007.

Hams, E., Aviello, G., Fallon, P.G., 2013. The schistosoma granuloma: friend or foe? Front. Immunol. 4, 89. https://doi.org/10.3389/fimmu.2013.00089.

Jones, M.K., Gobert, G.N., Zhang, L., Sunderland, P., McManus, D.P., 2004. The cytoskeleton and motor proteins of human schistosomes and their roles in surface maintenance and host-parasite interactions. Bioessays 26, 752–765. https://doi.org/10.1002/bies.20058.

Katz, N., Coelho, P.M.Z., 2008. Clinical therapy of schistosomiasis mansoni: the Brazilian contribution. Acta Trop 108, 72–78. https://doi.org/10.1016/j.actatropica.2008.05.006.

Lorsuwannarat, N., Saowakon, N., Ramasoota, P., Wanichanon, C., Sobhon, P., 2013. The anthelmintic effect of plumbagin on *Schistosoma mansoni*. Exp. Parasitol. 133, 18–27. https://doi.org/10.1016/j.exppara.2012.10.003.

Mahmoud, M.R., El-Abhar, H.S., Saleh, S., 2002. The effect of Nigella sativa oil against the liver damage induced by *Schistosoma mansoni* infection in mice. J. Ethnopharmacol. 79, 1–11. https://doi.org/10.1016/S0378-8741(01)00310-5.

McManus, D.P., Dunne, D.W., Sacko, M., Utzinger, J., Vennervald, B.J., Zhou, X.N., 2018. Schistosomiasis. Nat. Rev. Dis. Primers 4, 13. https://doi.org/10.1038/s41572-018-0013-8.

Melman, S.D., Steinauer, M.L., Cunningham, C., Kubatko, L.S., Mwangi, I.N., Wynn, N.B., Mutuku, M.W., Karanja, D.M., Colley, D.G., Black, C.L., Secor, W.E., Mkoji, G.M., Loker, E.S., 2009. Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of *Schistosoma mansoni*. PLoS Negl. Trop. Dis. 3, e504. https://doi.org/10.1371/journal.pntd.0000504.

Melo, L.M., Perosso, J., Almeida, B.F., Silva, K.L., Somenzari, M.A., de Lima, V.M., 2014. Effects of P-MAPA immunomodulator on toll-like receptor 2, ROS, nitric oxide, MAPKp38 and IKK in PBMC and macrophages from dogs with visceral leishmaniasis. Int. Immunopharmacol. 18, 373–378. https://doi.org/10.1016/j.intimp.2013.12.012.

Mulvenna, J., Moertel, L., Jones, M.K., Nawaratna, S., Lovas, E.M., Gobert, G.N., Colgrave, M., Jones, A., Loukas, A., McManus, D.P., 2010. Exposed proteins of the*Schistosoma japonicum* tegument. Int. J. Parasitol. 40, 543–554. https://doi.org/10.1016/j.ijpara.2009.10.002.

Neves, B.J., Andrade, C.H., Cravo, P.V., 2015. Natural products as leads in schistosome drug discovery. Molecules 20, 1872–1903. https://doi.org/10.3390/molecules20021872.

Pellegrino, J., Oliveira, C.A., Faria, J., Cunha, A.S., 1962. New approach to the screening of drugs in experimental schistosomiasis mansoni in mice. Am. J. Trop. Med. Hyg. 11, 201–215. https://doi.org/10.4269/ajtmh.1962.11.201.

Ramirez, B., Bickle, Q., Yousif, F., Fakorede, F., Mouries, M.A., Nwaka, S., 2007. Schistosomes: challenges in compound screening. Expert. Opin. Drug. Discov. 2, S53–S61. https://doi.org/10.1517/17460441.2.S1.S53.

Reagan-Shaw, S., Nihal, M., Ahmad, N., 2008. Dose translation from animal to human studies revisited. FASEB J 22, 659–661. https://doi.org/10.1096/fj.07-9574LSF.

Sanderson, L., Bartlett, A., Whitfield, P.J., 2002. *In vitro* and *in vivo* studies on the bioactivity of a ginger (*Zingiber officinale*) extract towards adult schistosomes and their egg production. J. Helminthol. 76, 241–247. https://doi.org/10.1079/JOH2002116.

Santos, J.C., Batista, A.D., Vasconcelos, C.M.M., Lemos, R.S., Souza Junior, V.R., Dessein, A., Dessein, H., Montenegro, S.M.L., Lopes, E.P.A., Domingues, A.L.C., 2018. Liver ultrasound elastography for the evaluation of periportal fibrosis in schistosomiasis mansoni: a cross-sectional study. PLoS Negl. Trop. Dis. 12, e0006868 https://doi.org/10.1371/journal.pntd.0006868.

Santiago, M.E., Neto, L.S., Alexandre, E.C., Munari, D.P., Andrade, M.M., Somenzari, M. A., Ciarlini, P.C., de Lima, V.M., 2013. Improvement in clinical signs and cellular immunity of dogs with visceral leishmaniasis using the immunomodulator P-MAPA. Acta Trop 127, 174–180. https://doi.org/10.1016/j.actatropica.2013.04.005.

Schwartz, C., Fallon, P.G., 2018. *Schistosoma* "Eggs-Iting" the host: granuloma formation and egg excretion. Front. Immunol. 9, 2492. https://doi.org/10.3389/fimmu.2018.02492.

Silva, J.C.S.S., Bernardes, M.V.A.S., Melo., F.L., Sá., M.P.B.O., Carvalho., B.M., 2020. Praziquantel *versus* praziquantel associated with immunomodulators in mice infected with *schistosoma mansoni:* a systematic review and meta-analysis. Acta Trop 204, 105359. https://doi.org/10.1016/j.actatropica.2020.105359.

WHO, 2017. Integrating Neglected Tropical Diseases Into Global Health and development: Fourth WHO Report On Neglected Tropical diseases. World Health Organization, Geneva.

Xiao, S.H., Keiser, J., Chollet, J., Utzinger, J., Dong, Y., Endriss, Y., Vennerstrom, J.L., Tanner, M., 2007. *In vitro* and *in vivo* activities of synthetic trioxolanes against major human schistosome species. Antimicrob. Agents Chemother. 51, 1440–1445. https://doi.org/10.1128/AAC.01537-06.

Zwang, J., Olliaro, P.L., 2014. Clinical efficacy and tolerability of praziquantel for intestinal and urinary schistosomiasis-a meta-analysis of comparative and non-comparative clinical trials. PLoS Negl. Trop. Dis. 8, e3286. https://doi.org/10.1371/journal.pntd.0003286.