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ORIGINAL ARTICLE

Effect of phenyl vinyl sulphone cysteine protease inhibitor on *Schistosoma mansoni*: in vitro and in vivo experimental studies

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Abstract The present work aimed to study the effect of phenyl vinyl sulphone (PVS), a CPI, on different stages of Schistosoma (S.) mansoni in an in vitro culture study and in experimentally infected mice, compared to PZQ. As regards the in vitro study, different concentrations of PVS (1, 2, 4, 6, 8 and 10 µg/ml) and PZQ (1 µg/ml) were assessed by % worm mortality for schistosomula and adults, and hemoglobin degradation by schistosomula. In vivo study included 8 groups of mice. Intraperitoneal PVS, subgroup (a), and oral PZQ, subgroup (b), were assessed at different durations post infection (pi); at 1, 3, 5 and 7 weeks pi (groups I, II, III and IV, respectively). Infection, PVS, PZQ, and normal control groups (groups V–VIII) were included. The anti-schistosomal effects of PVS were assessed by parasitological, histopathological and haematological parameters. In in vitro study, PVS had a schistosomicidal effect in a concentration and time dependent manner, PVS showed 100% schistosomula mortality at day 2 and 92% adult worm mortality at day 5. Furthermore, PVS decreased hemoglobin degradation by schistosomula. In in vivo study, PVS showed a decrease in total worm burden and tissue egg load in intestine and liver with an increase in number of dead ova in intestine of mice. Furthermore, PVS resulted in a decrease in number, size and cellularity of hepatic granulomas and an increase in hemoglobin concentration.PVS was better than PZQ in reducing each of tissue egg count in intestine at 5 and 7 weeks pi, and hepatic granuloma size at 3, 5 and 7 weeks pi. These results suggest that PVS can be a promising chemotherapeutic agent in *Schistosoma mansoni* infection.

Keywords *Schistosoma mansoni* · Cysteine protease · Cysteine protease inhibitor · Phenyl vinyl sulphone · Praziquantel · In vitro · In vivo

Introduction

Schistosomiasis is a common disease in endemic areas of Sub-Saharan Africa, South America and Asia (Hinz et al. 2017). Praziquantel (PZQ) is the only drug of choice for treatment of human schistosomiasis due to its high efficacy, simple administration and competitive cost (Wang et al. 2012). Although PZQ is highly effective in killing adult worms, yet it has a poor efficacy against larvae and immature worms (Caffrey 2007, Danso-Appiah et al. 2013). Moreover, there have been stronger evidences of PZQ-resistance by *Schistosoma* (*S.*) *mansoni* (Pinto-Almeida et al. 2016).

The gut of schistosomula and adult worms of *S. mansoni* synthesize and secrete different cathepsins belonging to the cysteine protease family, that are involved in host hemoglobin degradation; a major source for the schistosome metabolism, growth, and in parasite development and reproduction (Correnti et al. 2005). These enzymes may function in membrane biogenesis or in intracellular protein

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turnover, besides their roles in the hemoglobin degradation (Dalton et al. 1995). Phenyl vinyl sulfone (PVS) was chosen as an example of the vinyl sulfones to study its effect on *S. mansoni* using in vitro culture technique, which was not studied before, as PVS effect on in vitro culture was studies with *Fasciola* only (Helmy et al. 2008). As well as in vivo parameters through different durations to assess its effect on both acute and chronic schistosomiasis.

The aim of the current work is to study the effect of a cysteine protease inhibitor; Phenyl vinyl sulfone (PVS) on different stages of *Schistosoma mansoni* in an in vitro culture study, and on the parasite subjected to PVS at different durations of *Schistosoma mansoni* in experimentally-infected mice, in comparison to the conventionally employed drug; praziquantel (PZQ).

Materials and methods

Parasite

Schistosoma mansoni cercariae were obtained from laboratory-bred infected *Biomphalaria alexandrina* snails in Schistosome Biological Supply Program (SBSP),Theodor Bilharz research institute (TBRI), Giza, Egypt.

Drugs

Phenyl vinyl sulphone (PVS) (Sigma, USA) and Praziquantel (PZQ) (EPICO, Egypt).

In vitro study

Ten *Schistosoma mansoni* schistosomula and 6 adult worms (3 males and 3 females) were maintained, separately, in RPMI-1640 medium (Neves et al. 2011). One mg of each of PVS and PZQ was dissolved in Dimethyl sulphoxide (DMSO)then diluted in 1 ml medium to make a working solution (Botros et al. 2009). Different concentrations of PVS (1, 2, 4, 6, 8 and 10 µg/ml) were applied to the wells in duplicates. They were assessed by dose response studies in the form of % worm mortality for schistosomula and adults (schistosomula and adult worms showing no signs of motility for 1 min, were considered dead), which were monitored every 24 h (hrs) for 96 h under inverted microscope ×30 (Abdulla et al. 2009; Botros et al. 2009)and growth inhibition studies in the form of hemoglobin degradation (in the form of black gut) by schistosomula, where the schistosomula were monitored every 24 h for an 8-day period (Wasilewski et al. 1996). Positive and negative controls (PZQ at 1 µg/ml and DMSO, respectively) were also studied (Neves et al. 2011).

In vivo study

The study was performed on 72 female Swiss albino mice, 6–8 weeks old, weighing 20–25 g, obtained and housed, fulfilling the animal ethical considerations at the SBSP, Theodor Bilharz research institute, Giza, Egypt. Mice were classified into the following groups:

Group I	[Infected mice treated 1 week post-infection (pi)] included 12 mice equally divided into: subgroup (a): Infected-PVS treated mice, and subgroup (b): Infected-PZQ treated mice.
Group II	(Infected -mice treated 3 weeks pi) included 12 mice equally divided into: subgroup (a): Infected-PVS treated mice, and subgroup (b): Infected-PZQ treated mice.
Group III	(Infected mice treated 5 weeks pi) included 12 mice equally divided into: sub group (a): Infected-PVS treated mice, and subgroup (b): Infected-PZQ treated mice.
Group IV	(Infected mice treated 7 weeks pi)included 12 mice equally divided into: subgroup (a): Infected-PVS treated mice, and subgroup (b): Infected-PZQ treated mice.
Group V	(Infection control):6 infected non-treated mice.
Group VI	(PVS control):6 non-infected PVS treated mice.
Group VII	(PZQ control):6 non-infected PZQ treated mice.
Group VIII	(Normal control): 6 non-infected non-treated apparently healthy mice.

Mice were infected by 60 ± 10 *S. mansoni* cercariae by the subcutaneous injection route (Peters and Warren 1969) at SBSP.

Each drug was dissolved in its suitable solvent; PVS powder was freshly prepared by being dissolved in sterile distilled water at a concentration of 5 mg/ml and heated at 60 °C till completely dissolved. A 600 mg tablet of PZQ was ground and suspended in 2% Cremophor El. Then each drug was administered through its suitable route according to its pharmacokinetics; the administered dose of PVS was 50 mg/kg/mouse, once daily by intraperitoneal injection for 7 consecutive days (Abdulla et al. 2007). While, PZQ was freshly prepared before oral administration at a dose of 500 mg/kg/mouse, once daily for two consecutive days using nasogastric feeding tube (Keiser et al. 2006; Abaza et al. 2013). All mice were sacrificed at 10 weeks pi.

The anti-schistosomal effects of PVS were assessed by:

1. *Parasitological parameters* Small intestine and liver from each mouse were examined for total worm burden by sucking the worms from their tissues (Smithers and Terry 1965),tissue egg load in small

intestine and liver (Cheever 1968) and oogram pattern (Pellegrino and Faria 1965) where ova were classified according to their stages of development into: mature (fully developed miracidia), immature (small embryos occupying 1/3, 1/2, 2/3 or the whole space of the egg shell)or dead (eggs appear semitransparent, granular and dark showing a clear longitudinal half).

2. *Histopathological parameters* Part of liver was embedded in paraffin blocks to prepare slides from sections of 5 µm thickness followed by staining with Hematoxylin and Eosin (Hx and E) stain for detection of egg-induced granulomatous cellular reaction (Druray and Wallington 1980).Microscopic examination of previously prepared liver sections stained with Hx and E stain to determine the granuloma number and size, according to the following criteria (Jacobs et al.1997):
    1. Single egg granulomas were selected for diameter measurements.
    2. The greatest diameter and its perpendicular diameter were measured, by using a trinocular microscope with mounted camera, and the mean of both diameters was considered granuloma diameter.
    3. The mean size of granulomas for each group was calculated.
    4. Mean number of granulomas/low power field (×10 objective) was calculated in each group.
3. *Haematological parameter* Detection of hemoglobin concentration for each mouse followed by the detection of mean hemoglobin concentration for each group (Feldmann et al. 2000).
4. *Statistical study* The collected data were introduced to a personal computer using Statistical Package for Social Science (SPSS 18.0.1 for Windows; SPSS Inc, Chicago, IL). Descriptive statistics included: mean, standard deviation (±SD) for parametric numerical data, percentage of non-numerical data. Analytical statistics was done using the Mann–Whitney Test (U test) to assess the statistical significance of the difference of a variable between two study groups. p value less than or equal 0.05 was considered significant.IC_{50} (Half maximal inhibitory concentration) was also calculated (Sebaugh 2011).

Results

In vitro study

Dose–response studies

10 *Schistosoma mansoni* schistosomula and 6 adult worms (3 males and 3 females) were maintained were subjected to different concentrations of PVS (1, 2, 4, 6, 8 and 10 µg/ml) then all schistosomula and adult worms in all wells were counted (Neves et al. 2011). The 10 µg/ml dose of PVS resulted in 90% schistosomula mortality by day 1 which was higher than that by PZQ (80%), reaching 100% mortality rate by day 2, the IC_{50} of PVS was 7.5 µg/ml at day 1, 4 µg/ml at day 2 and 2.5 µg/ml at day 3 (Fig. 1; Table 1). Moreover, PVS resulted in 92% adult worms mortality by day 5 reaching 100% by day 6, the IC_{50} of PVS was 6 µg/ml at day 4 and 3.9 µg/ml at day 5 (Fig. 2; Table 2).

Growth inhibition studies

In the current study, the effect of different concentrations of PVS on *S. mansoni* schistosomula hemoglobin degradation in vitro was studied. Results showed concentration dependent arrest of hemoglobin degradation in schistosomula treated with PVS as determined by lack of black pigmentation of the gut (Fig. 3).

In vivo study

Parasitological parameters

Total worm burden As regards the in vivo study in the present work, PVS decreased total worm burden when given at 1, 3, 5 and 7 wks pi with non-significant difference ($p > 0.05$) when compared to infection control. A significant decrease ($p \leq 0.05$) in total worm burden was recorded in mice treated with PZQ at 5 and 7 wks pi compared to infection control (Fig. 4).

Tissue egg count/gm intestine and liver In the current work, PVS given at 1, 3, 5 and 7 wks pi showed significant decrease ($p \leq 0.05$) in count of eggs/gm intestine and liver when compared to infection control (Figs. 5 and 6).

Oogram pattern In the present study, the effect of PVS on the oogram pattern of *S. mansoni* eggs was assessed. At

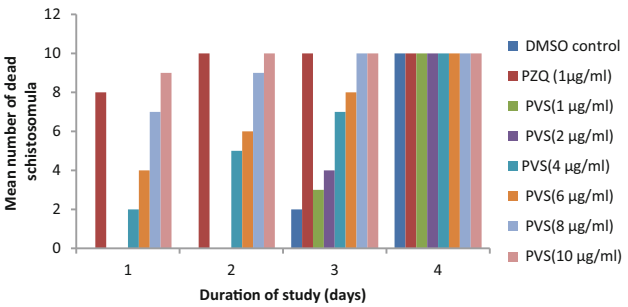

Fig. 1 Bar chart illustrating the effect of different concentrations of PVS on the mortality of schistosomula in comparison to PZQ and DMSO controls. The IC_{50} of PVS was 7.5 µg/ml at day 1, 4 µg/ml at day 2 and 2.5 µg/ml at day 3

Table 1 Mortality rate of *S. Mansoni* schistosomula (% worm mortality)

Tested compounds		% Schistosomula mortality			
		Day 1	Day 2	Day 3	Day 4
PVS	1 μg/ml	0	0	30	100
	2 μg/ml	0	0	40	100
	4 μg/ml	20	50	70	100
	6 μg/ml	40	60	80	100
	8 μg/ml	70	90	100	100
	10 μg/ml	90	100	100	100
DMSO control		0	20	100	100
PZQ (1 μg/ml)		80	100	100	100

1, 3 and 5 wks pi, PVS showed an oogram pattern similar to that of infection control. Moreover, at 7 wks pi, PVS showed a significant increase ($p \leq 0.05$) in mean number of dead ova compared to infection control (Table 3).

Histopathological parameter

In the current study giving each of PVS and PZQ at early durations of infection (at 1 and 3 wks pi) resulted in a nearly similar histopathological picture as infection control (Figs. 7, 8 and 9). There was cloudy swelling in liver cells with cellular granuloma made up of histiocytes, lymphocytes and scattered fibroblasts with bilharzial pigment in Von-Kupffer cells. Also, at 1 and 3 wks pi there was a recorded significant decrease ($p \leq 0.05$) in mean hepatic granuloma size when using each of PVS and PZQ compared to infection control, with PVS resulting in smaller granuloma size than PZQ when each was given at 3 wks pi ($p \leq 0.05$). Moreover, there was a recorded significant decrease ($p \leq 0.05$) in mean hepatic granuloma number at 3 wks pi using each of PVS and PZQ compared to infection control (Tables 4 and 5).

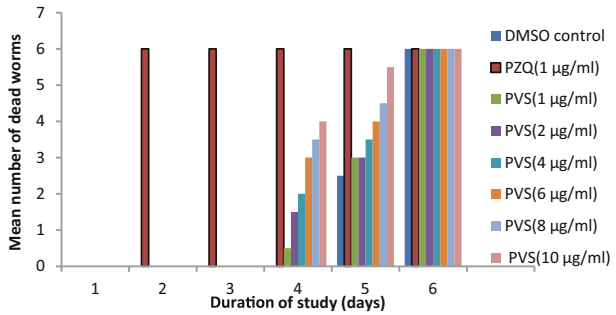

Fig. 2 Bar chart illustrating the effect of different concentrations of PVS on the viability of *S. mansoni* adult worms in comparison to PZQ and DMSO controls. The IC_{50} of PVS was 6 μg/ml at day 4 and 3.9 μg/ml at day 5

1. Histopathological examination of liver tissue (Fig. 7):
2. Hepatic granuloma number and size.
   a. Hepatic granuloma number.
   b. Hepatic granuloma size.

Hematological parameter

In the present work, the study of the effect of PVS on hemoglobin concentration in *S. mansoni* infected mice revealed that PVS increased mean hemoglobin

Table 2 Mortality rate of *S. mansoni* adult worms (% worm mortality)

Tested compounds		% Adult worms mortality					
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
PVS	1 μg/ml	0	0	0	8	50	100
	2 μg/ml	0	0	0	25	50	100
	4 μg/ml	0	0	0	33	51	100
	6 μg/ml	0	0	0	50	67	100
	8 μg/ml	0	0	0	51	75	100
	10 μg/ml	0	0	0	67	92	100
DMSO control		0	0	0	50	100	100
PZQ (1 μg/ml)		0	100	100	100	100	100

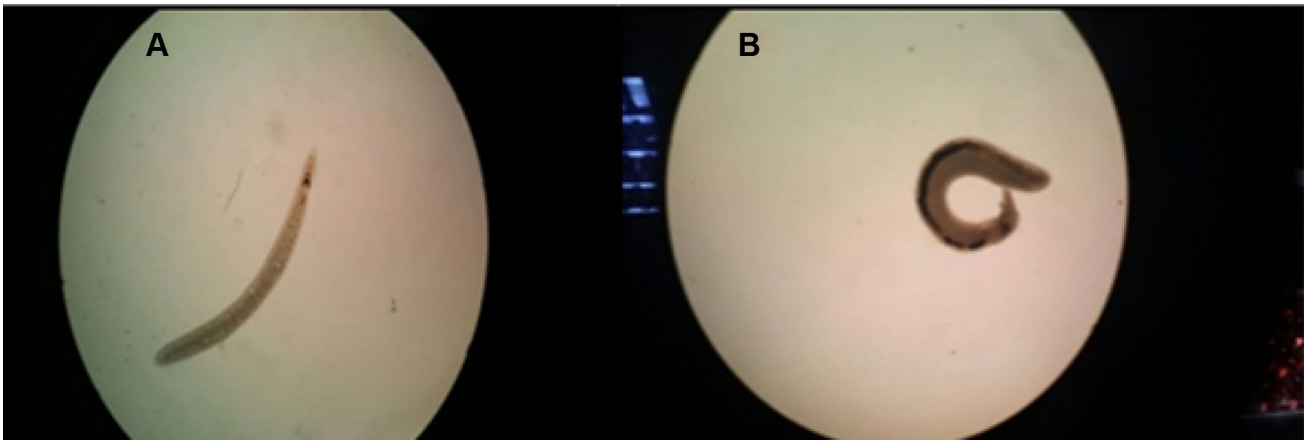

Fig. 3 **a** Schistosomula in 10 µg/ml PVS showing absence of the black pigmentation of the gut (×4 objective). **b** Schistosomula in 1 µg/ml PZQ showing the black pigmentation of the gut (×4 objective)

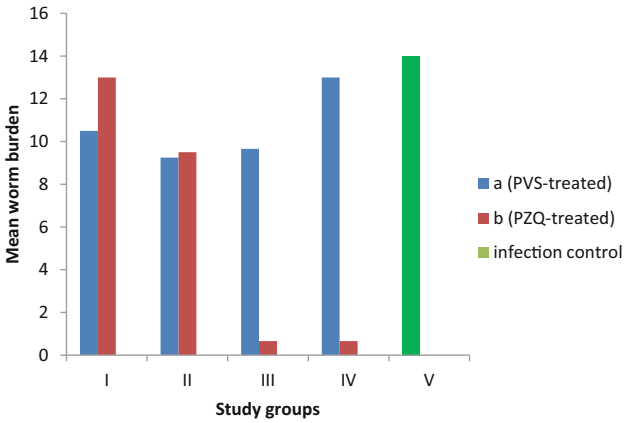

Fig. 4 Bar chart illustrating mean total worm burden among different study groups. A decrease in mean total worm burden in each of subgroup a in groups I, II, III and IV in comparison to group V was recorded. Subgroup a in group I showed decrease in mean number of worms than subgroup b of the same group

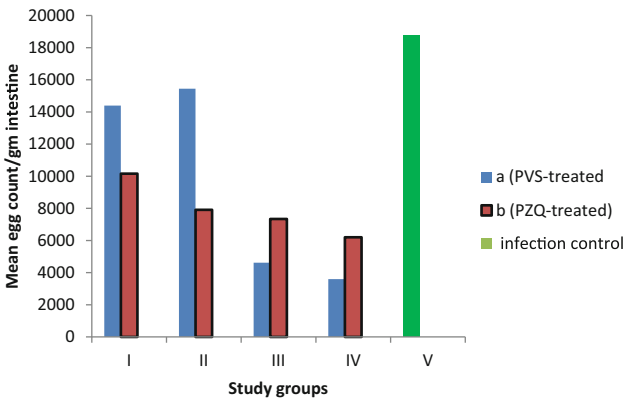

Fig. 5 Bar chart illustrating mean egg count/gm intestine among different study groups. A decrease in mean egg count/gm intestine in subgroup a of groups I, II, III and IV compared to group V was recorded. Subgroup a in each of group III and IV showed decrease in mean egg count than subgroup b of same groups

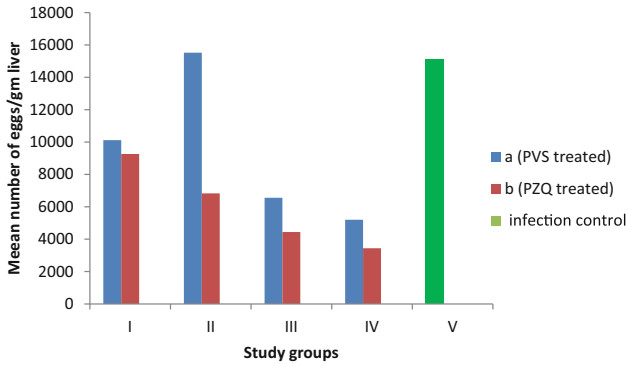

Fig. 6 Bar chart illustrating mean count of eggs/gm liver among different study groups showing a decrease in subgroup a in each of groups I, II, III and IV in comparison to group V

concentration when given at different durations of infection resulting in a significant ($p \leq 0.05$) increase in mean hemoglobin concentration when given at each of1and 5wk pi compared to infection control.PVS given at different durations post infection showed non-significant difference ($p > 0.05$) in mean hemoglobin concentration when compared to PZQ treated- mice (Table 6).

Discussion

The present work was done to study the effect of different concentrations of PVS, a CPI, on *S. mansoni* schistosomula and adults in an in vitro study and on the parasite subjected to PVS at different durations of experimental *S. mansoni* infection.

Currently, as regards in vitro study, the *S. mansoni* schistosomula and adult worms were subjected to different concentrations of PVS (1, 2, 4, 6, 8 and 10 µg/ml) (Neves et al. 2011). The 10 µg/ml dose of PVS resulted in 90% schistosomula mortality by day 1 which was higher than

Table 3 Results of oogram pattern of *S. mansoni* eggs among different study groups

Group and subgroup (6 mice each)	Mature egg (mean ± SD)	Immature egg (mean ± SD)	Dead egg (mean ± SD)
Ia (PVS treated 1 wk pi)	42.75 ± 1.708#	52.00 ± 2.44#	5.25 ± 1.258#
Ib (PZQ treated 1 wk pi)	43.00 ± 2.44	52.50 ± 2.88	4.50 ± 1.00
IIa (PVS treated 3 wk pi)	45.50 ± 4.20#	52.00 ± 4.24#	5.00 ± 0.82#
IIb (PZQ treated 3 wk pi)	43.25 ± 2.87	52.00 ± 2.45	4.75 ± 1.71
IIIa (PVS treated 5 wk pi)	42.33 ± 2.08	52.33 ± 2.517	5.33 ± 1.53
IIIb (PZQ treated 5 wk pi)	49.66 ± 4.509	5.33 ± 0.57	45.00 ± 5.00
Iva (PVS treated 7 wk pi)	46.00 ± 3.61	41.66 ± 2.88*	12.33 ± 2.52*
IVb (PZQ treated 7 wk pi)	10.33 ± 12.85	1.66 ± 2.88	88.0 ± 15.7
V (Infection control)	44.25 ± 4.03	53.25 ± 2.363	4 ± 0.816

* Significant difference in comparison to infection control ($p \leq 0.05$)

Non significant difference in comparison to PZQ treated mice at same duration ($p > 0.05$)

that by PZQ (80%), reaching 100% mortality rate by day 2, the half maximal inhibitory concentration (IC_{50}) of PVS was 7.5 µg/ml at day 1,4 µg/ml at day 2 and 2.5 µg/ml at day 3 (Fig. 1; Table 1). Moreover, PVS resulted in 92% adult worms mortality by day 5 reaching 100% by day 6, the IC_{50} of PVS was 6 µg/ml at day 4 and 3.9 µg/ml at day 5 (Fig. 2; Table 2). This goes well with the results of Fahmy and Helmy (2007) who reported that 300 ppm PVS caused immediate death of *S. mansoni* adults. Similarly, Farid et al. (2013b) studied the effect of other CPIs on the viability of *S. mansoni* adults and their results showed that death started 1 h after exposure to 500 ppm of FMK, VS and sodium-nitro-prussid with percent worm mortality 75, 70 and 60%, respectively and reaching 100% mortality after 3 h. The death of PZQ treated worms started from day 2 with 100% worm mortality. This goes well with previous studies which reported that PZQ exerts schistosomicidal activity in vitro on schistosomula and adults of *S. mansoni* (Cioliand Pica-Mattoccia 2002; Neves et al. 2011).

In the current study, the effect of different concentrations of PVS on *S. mansoni* schistosomula hemoglobin degradation in vitro was studied. Results showed concentration dependent arrest of hemoglobin degradation in schistosomula treated with PVS as determined by lack of black pigmentation of the gut (Fig. 3). These results were in context with Wasilewski et al. (1996) who revealed that CPIs produced a dramatic arrest of hemoglobin degradation in vitro by schistosomula suggesting that the death of schistosomula in their study, was due to inhibition of CPs and not due to toxic effect of the inhibitor. Also, Shenai et al. (2003) tested the effect of VSs on *Plasmodium falciparum* and showed that inhibition of parasite development was accompanied by the appearance of darkly stained, swollen food vacuoles, which are indicative of a block in hemoglobin hydrolysis and blocking of food vacuole hemoglobinases.

As regards the in vivo study in the present work, a significant decrease ($p \leq 0.05$) in total worm burden was recorded in mice treated with PZQ at 5 and 7 wks pi compared to infection control (Fig. 4) which goes well with the known potency of PZQ against schistosome adult worms (Xiao et al. 1985, Issa 2007). PVS given at 1 and 3 wks pi showed a non-significant decrease ($p > 0.05$) in total number of worms when compared to PZQ treated-mice at the same durations, which is on the contrary to the results of Farid et al. (2013b) using other CPIs at 3 wks pi. However, at 5 and 7 weeks pi the PZQ treated-mice showed significant decrease ($p \leq 0.05$) in total number of worms compared to PVS given at the same durations. These results are similar to another study where PZQ and PVS were given to mice at 6 wks pi (Abaza et al. 2013).

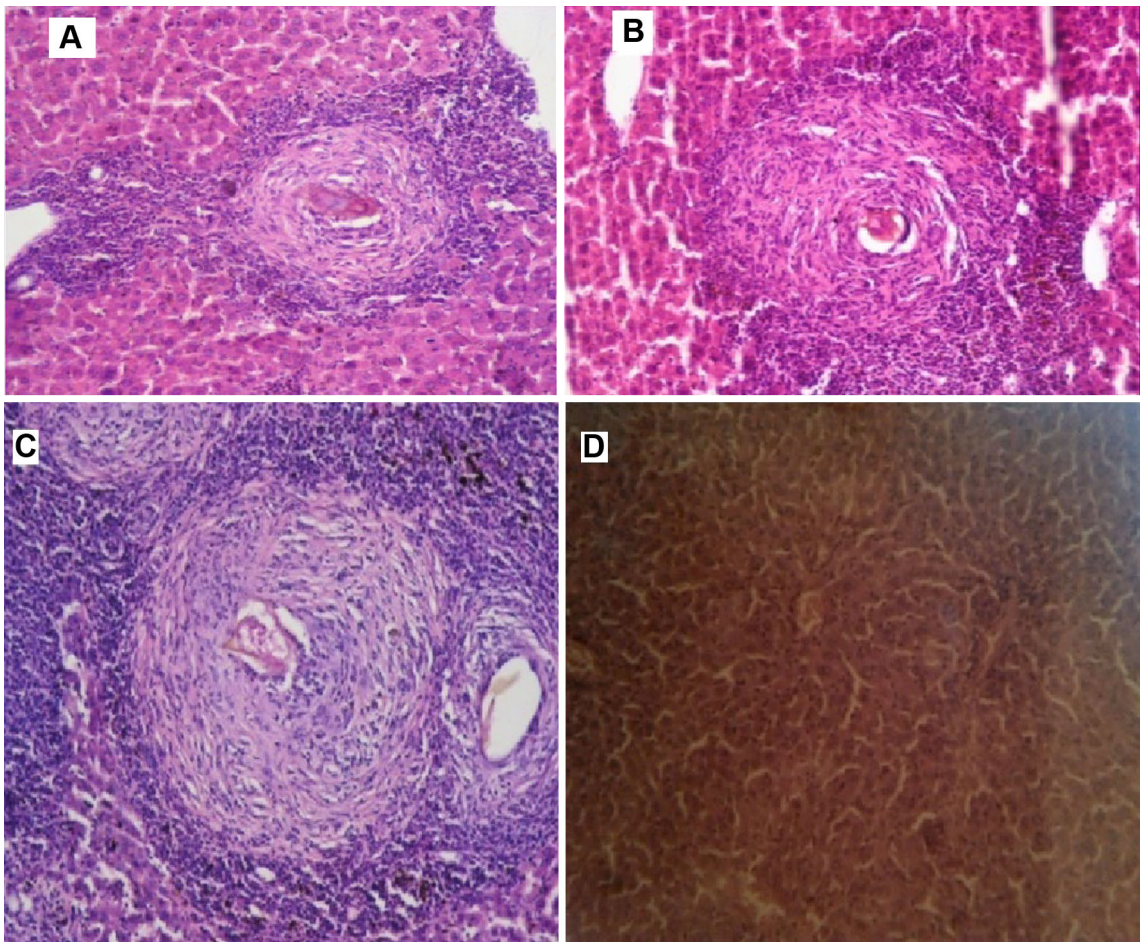

Fig. 7 Hepatic granuloma in groups of study**. a** Subgroup IIa showing a single dead partially calcified *S. mansoni* ova in the center of a well circumscribed granuloma made up of fibroblasts with lymphocytes mainly seen at the periphery. Perigranulomatous tissue shows expansion of portal tracts by lymphocytic infiltrate along with bile duct injury and focal interface hepatocytes (Hx and E stain ×400). **b** Subgroup IIb showing a single dead partially calcified *S. mansoni* ova in the center of a partially circumscribed granuloma made up of histiocytes and fibroblasts with intervening collagen, lymphocytes mainly seen at the periphery (Hx and E stain ×400). **c** Infection control group (V) showing granuloma made up of histiocytes and few fibroblasts with lymphocytes mainly the periphery, a viable *S. mansoni* ova is seen, perigranulomatous tissue shows lymphocytic infiltrate along with bilharzial pigment in Von-Kupffer cells (Hx and E stain ×400). **d** Normal control group (VI) (Hx and E stain ×400)

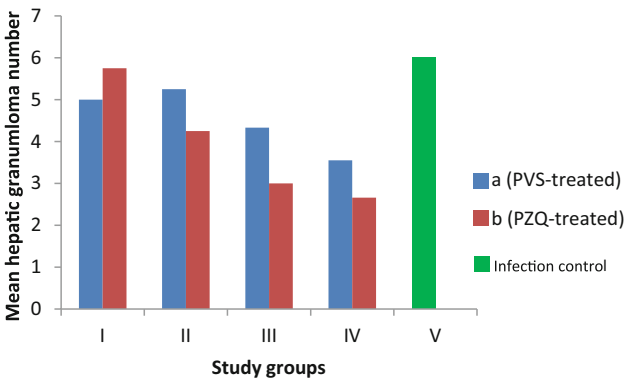

Fig. 8 Bar chart illustrating mean hepatic granuloma number among different study groups showing a decrease in mean hepatic granuloma number in subgroup a of each of group I, II, III and IV compared to group V

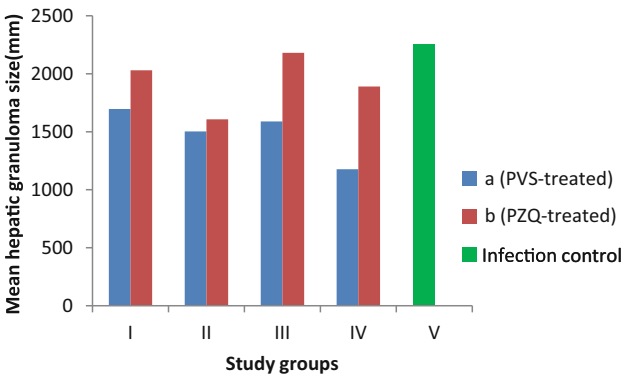

Fig. 9 Bar chart illustrating mean hepatic granuloma size among different study groups. A decrease in mean hepatic granuloma size in subgroup a of each of group I, II, III and IV compared to group V, and compared to subgroup b of the same groups

Table 4 Statistical analysis of hepatic granuloma number among different study groups

Group and subgroup (6 mice each)	Mean ± SD
Ia	5.0 ± 0.57#
(PVS treated 1wk pi)	
Ib	5.75 ± 0.95
(PZQ treated 1wk pi)	
IIa	5.25 ± 0.95*#
(PVS treated 3wk pi)	
IIb	4.25 ± 0.9
(PZQ treated 3wk pi)	
IIIa	4.33 ± 0.57*#
(PVS treated 5 wk pi)	
IIIb	3.0 ± 1.0
(PZQ treated 5 wk pi)	
Iva	3.66 ± 0.52*#
(PVS treated 7 wk pi)	
IVb	2.66 ± 0.57
(PZQ treated 7 wk pi)	
V	6.0 ± 0.816
(Infection control)	

* Significant difference in comparison to infection control ($p \leq 0.05$)

Non significant difference in comparison to PZQ treated mice at same duration ($p > 0.05$)

Table 5 Statistical analysis of hepatic granuloma size (in mm) among different study groups

Group and subgroup (6 mice each)	Mean ± SD
Ia	1696.75 ± 296.8*#
(PVS treated 1wk pi)	
Ib	2030.25 ± 162.9
(PZQ treated 1wk pi)	
IIa	1502.75 ± 9.03*•
(PVS treated 3wk pi)	
IIb	1606.7 ± 6.40
(PZQ treated 3wk pi)	
IIIa	1589.33 ± 6.66*
(PVS treated 5 wk pi)	
IIIb	2180.67 ± 7.02
(PZQ treated 5 wk pi)	
Iva	1176.67 ± 7.64*
(PVS treated 7 wk pi)	
IVb	1890 ± 4.0
(PZQ treated 7 wk pi)	
V	2256 ± 11.34
(Infection control)	

* Significant difference in comparison to infection control ($p \leq 0.05$)

Non significant difference in comparison to PZQ treated mice at same duration ($p > 0.05$)

• Significant difference in comparison to PZQ treated mice at same duration ($p \leq 0.05$)

In the current work, PVS given at 1, 3, 5 and 7 wks pi showed significant decrease ($p \leq 0.05$) in count of eggs/gm intestine and liver when compared to infection control (Figs. 5 and 6). In this context, Abaza et al. (2013) also found that PVS treated mice showed decrease in mean tissue egg load/gm liver and intestine in comparison to infected non treated control mice in their study.

In the present study, the effect of PVS on the oogram pattern of *S. mansoni* eggs was assessed (Table 3). At 1, 3 and 5 wks pi, PVS showed an oogram pattern similar to that of infection control. To the contrary of Farid et al. (2013a) who by studying 3 CPIs; VS, FMK and sodium nitroprussid given at 3 wks and 5 wks pi reported an increase in mean number of dead ova in comparison to infected non treated mice, in their study. Moreover, at 7 wks pi, PVS showed a significant increase ($p \leq 0.05$) in mean number of dead ova compared to infection control. These results were in context with Abaza et al. (2013) who revealed that PVS given at 6 wks pi showed a decrease in mean number of immature ova and increase in mean number of dead ova in comparison to infected non treated group in their study.

In the current study giving each of PVS and PZQ at early durations of infection (at 1 and 3 wks pi) resulted in a nearly similar histopathological picture as infection control (Figs. 7, 8 and 9) (Table 4 and 5). There was cloudy swelling in liver cells with cellular granuloma made up of histiocytes, lymphocytes and scattered fibroblasts with bilharzial pigment in Von-Kupffer cells. Also, at 1 and 3 wks pi there was a recorded significant decrease ($p \leq 0.05$) in mean hepatic granuloma size when using each of PVS and PZQ compared to infection control, with PVS resulting in smaller granuloma size than PZQ when each was given at 3 wks pi ($p \leq 0.05$). Moreover, there was a recorded significant decrease ($p \leq 0.05$) in mean hepatic granuloma number at 3 wks pi using each of PVS and PZQ compared to infection control (Tables 4 and 5). The above mentioned findings go well with the results of Farid et al. (2013a) who found that VS, a CPI, given to mice at 3 wks pi, showed similar histopathological picture with a decrease in hepatic granuloma size and number compared to infection control in their study. They deduced that their detected VS effect can be attributed to a mild suppression of the host immune system. The results of histopathological examination using PZQ in the present study goes well with those of El Beshbishi et al. (2013) when using PZQ 3 and6 wks pi. The decreased granuloma size in PZQ treated mice, in the present study, can be attributed to the fact that hepatic granulomas shrink progressively after using a curable drug (Morsy 2009).

Table 6 Statistical analysis of mean hemoglobin concentration (gm/100 ml) among different study groups

Group and subgroup (6 mice each)	Mean ± SD
Ia (PVS treated 1wk pi)	13.5 ± 0.57*#
Ib (PZQ treated 1wk pi)	15.75 ± 1.5*
IIa (PVS treated 3wk pi)	10.75 ± 1.7#
IIb (PZQ treated 3wk pi)	13.25 ± 2.98*
IIIa (PVS treated 5 wk pi)	11.66 ± 2.08*#
IIIb (PZQ treated 5 wk pi)	15.0 ± 2.64*
Iva (PVS treated 7 wk pi)	9.66 ± 1.52#
IVb (PZQ treated 7 wk pi)	12.33 ± 2.08*
V (Infection control)	8.0 ± 1.0
VI (PVS control)	13.5 ± 1.1
VII (PZQ control)	12.6 ± 1.5
VIII (Normal control)	12.5 ± 0.5

* Significant difference in comparison to infection control ($p \leq 0.05$)

Non significant difference in comparison to PZQ treated mice at same duration ($p > 0.05$)

In the present work, the study of the effect of PVS on hemoglobin concentration in *S. mansoni* infected mice revealed that PVS increased mean hemoglobin concentration when given at different durations of infection resulting in a significant ($p \leq 0.05$) increase in mean hemoglobin concentration when given at each of1and 5wk pi compared to infection control (Table 6). The recorded increase in hemoglobin concentration induced by PVS probably reflects the inhibitory effect of PVS on the *S. mansoni* CPs. Vermiere et al. (2012) studied the effect of K11777, a CPI, against *Ancylostoma caninum* infected hamsters and reported that it reversed a severe decrease in blood hemoglobin levels. In the present work, PZQ induced a significant increase ($p \leq 0.05$) in mean hemoglobin concentration when given at different durations post infection compared to infection control, which goes well with Tohon et al. (2008) who reported that the use of PZQ was followed by a significant reduction of anemia. Meanwhile, PVS given at different durations post infection showed non-significant difference ($p > 0.05$) in mean hemoglobin concentration when compared to PZQ treated- mice.

It is concluded from the present study that PVS has a schistosomicidal effect in a concentration and time dependent manner with a possible anti-immonopathological role during *Schistosoma mansoni* infection. PVS was effective in killing *S. mansoni* schistosomula and adult worms in in vitro study. Moreover, PVS decreased hemoglobin degradation by schistosomula. As regards in vivo study, PVS showed a decrease in total worm burden and tissue egg load in intestine and liver with an increase in number of dead ova in intestine and resulted in a decrease in number, size and cellularity of hepatic granulomas. Furthermore, PVS was better than PZQ in reducing each of tissue egg count in intestine, and hepatic granuloma size.

Compliance with ethical standards

Conflict of interest We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome. We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

Ethical approval The study was approved by the Research Ethics Committee, Faculty of Medicine, Ain Shams University. All the animal experiments were performed according to the rules and regulation of the Animal Ethics rules, Ain-Shams University, Cairo, Egypt.

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