Full Length Research Paper

Determination of effective praziquantel dose in different mouse strains: BALB/c and Swiss mice in treatment of *Schistosoma mansoni*

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Schistosomiasis is a parasitic disease, second to malaria, affecting human's tropics and sub-tropics. The disease condition varies in severity depending on parasite species and strain, organ system infected, geographical region, genetic constitution of the individual and nutritional status. The drug praziquantel has been the choice of drug for the treatment of schistosomisias; however, the effective dose, 450 mg/kg body weight, which is currently being used, is not able to clear the worms completely. This work sought to determine the effective dose of praziquantel in different mouse strains of which the results can be applied in human treatment. Experimental groups comprising of twelve mice and eighteen for the infected control groups were designed for both BALB/c and Swiss mice. At four weeks post infection, mice were treated with varying dosages of praziquantel namely PZQ 1350, PZQ 900, PZQ 450 mg/kg body weight. At week 6, all mice were perfused to recover adult worms. Gross pathology and histopathology of the liver tissue were examined. Serum samples were collected to determine immunological responses in all the groups at week 4 and 6. Schistosomule soluble protein (SSP) and schistosomule warm antigen preparation (SWAP) specific antibody ELISA were done. Results indicated that in the experimental groups PZQ 1350 mg/kg body weight had few numbers of worms recovered in BALB/c and Swiss mice, that is, 30.30 and 34.08%, respectively, and a high worm reduction, that is, 69.70 and 65.92% respectively. The SSP and SWAP specific IgG responses were high due to synergistic effect between the drug and immune responses. Granuloma formation was greatly reduced in PZQ 1350 mg/kg body weight group in comparison to other treatments. The findings of this study imply that the higher the dosage of praziquantel, the more the protection against *Schistosoma mansoni* infection, since PZQ 1350 indicated better responses in worm recovery, worm reduction, immunological response and pathology compared to other dosages. These results may be incorporated into the design of a more effective dose; however, the toxicity of the high dose should be investigated. The findings also indicate that Swiss mouse was a better permissive host than BALB/c, as it allowed more parasites to mature instead of destroying them. Hence, it is a better model in schistosomiasis experimental studies.

Key words: Schistosomiasis, praziquantel, mice, pathology.

INTRODUCTION

Schistosoma mansoni occurs in Africa, Madagascar, the Arabian Peninsula, South America and the Caribbean region (Rollinson and Southgate, 1987). *S. mansoni* can be treated by oxamniquine and praziquantel, though the latter is preferred since it is principally safe and relatively cheap (Mutapi, 2001; WHO, 2001).

Praziquantel is an acylated quinoline-pyrazine (piperazine). Chemically piperazine is 2-cyclohexylcarbonyl-1, 2,3,6,7, 11b-hydropypyrazino {2, 1-a} isoquinolin-4-one). Only the enantiomer is active. At low concentrations *in vitro*, the drug appears to impair the function of the worms. At higher concentrations *in vitro*, praziquantel increases the contraction (irreversibly at very high concentrations) of the worm's strobilla (chain of proglottids). Also, praziquantel causes irreversible focal vacuolization with subsequent cestodal disintegration at specific sites of the cestodal integument (Ali, 2006). The main control strategy in schistosomiasis is the treatment of infected people with anthelmintic drugs and principally the use of praziquantel. Studies have shown that treatment with praziquantel damages the tegument of schistosomes (Andrew, 1985), exposing worm antigens (Fallon and Doenhoff, 1995) to the immune system. This action induces both humoral and cellular responses resulting in the death of the worm (Woolhouse and Hagan, 1999). It has also been shown that the interaction between praziquantel and schistosomes results in the alteration of host-parasite-specific immune responses.

Praziquantel kills adult worms and mature eggs but does not directly affect immature worms (Mutapi, 2001). The aim of the research was to determine the effective dose of praziquantel for treating *Schistosoma mansoni* infection in two mouse strains because the current dose 450 mg/kg b. w. (Farah et al., 2001) is not able to destroy the worms to a minimum number.

MATERIALS AND METHODS

Parasite and intermediate hosts

S. mansoni eggs were obtained from faecal material of infected olive baboon that were maintained at the Institute of Primate Research, IPR, Kenya. The eggs were hatched and 5 to 8 miracidia were used to infect each fresh water snails, *Biomphalaria pfeifferi.* The snails were maintained at snail facility at IPR, Kenya.

Animals

BALB/c and Swiss albino mice, 7 weeks old (at the commencement of the experiment) were maintained at Institute of Primate Research (IPR) laboratories in Kenya. The mice were housed at a temperature of about 25ºC with 12 h of light /12 h darkness photoperiod and fed on rodent pellets (Laboratory chow, Unga Feed ® Co.) and water *ad libitum.*

Experimental design

For each of the two strains of mice, BALB/c and Swiss, the following groups were used for experiments: Infected control (IC) mice infected with 250 *S. mansoni* cercariae and treated with distilled water. Experimental groups mice infected with 250 *S.*

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mansoni cercariae and treated with praziquantel (biltricide) PZQ 1350, PZQ 900, PZQ 450 receiving PZQ doses of 1350, 900, 450 mg/kg b. w., respectively. All infected groups (experimental and infected control) were infected with the same batch of *S. mansoni* cercariae. A schematic representation of the experimental design is given in Table 1.

Infection and challenge

Infected snails were shed and cercariae quantified for infection and challenge. During infection and challenge, mice were anaesthetized with a mixture of Rompun and Ketamine (Agrar, Holland) at 0.02 ml/30 g body weight via intraperitoneum. Mice received 250 normal *S. mansoni* cercariae. Mouse abdomen was shaved and a metal ring 1 cm in diameter was placed on the wet shaved area. Parasite suspension was placed in the metal ring. Parasites were given 30 min to penetrate.

Treatment

Praziquantel (Biltricide®, Bayer, Germany) tablets were ground into powder and then dissolved in distilled water dose of 450 mg/kg b. w., 900 mg/kg b. w. and 1350 mg/kg b. w. were given orally to each mouse (12).

Serum preparation

Blood was collected from anaesthetized mice using the heart puncture technique. At each specified sampling point, blood from a particular group was pooled. Serum was prepared from the pooled blood and stored at -20°C for analysis.

Preparation of antigen

18 h soluble larval antigen (SSP) was prepared following artificial transformation of *S. mansoni* cercariae and separation of heads from tails on a discontinuous Percoll gradient. The schistosomules were cultured at 37°C, 5% CO_2 in complete medium (RMPI 1640, 0.2 mg/ml gentamycin, 1% glutamine (2 mM); 1% 2-mercaptoethanol (5×10^{-5} M), fortified with 10% foetal calf serum) under sterile conditions for 18 h. They were then pelleted and washed two times in PBS and sonicated (24 kHz, 16 mm amplitude, 10 min) before centrifugation at 100,000 g for 1 h to obtain the soluble fraction. The protein content was determined and the solution was UV-sterilized and stored at -70°C.

Soluble worm antigen preparation (SWAP) was prepared from 5-week-old worms recovered from baboons. A commercially prepared concanavalin A (Con A; Sigma Co.) was dissolved in sterile PBS to make a concentration of 1 mg/ml. The solution was sterilized by nalgene disposable filter (Nalgene Co., USA).

Antibody assay

ELISA plate (Nunc-Immuno™ plate Marxi Sorp™ surface, Denmark) was coated with 50 μl of 5 μg/ml SSP and SWAP. It was incubated overnight at 4°C to allow the antigen to bind. The excess antigen was dispensed off on a blotting paper, then 100 μl of 3% BSA in PBS/tween 20 (0.05%) per well was added to block the free sites. The plate was incubated for one hour at 37°C and washed six times with PBS/tween 20 (0.05%). The serum samples were diluted 1:200 in 0.5%BSA/PBS/tween20. The blank (0.5% BSA/PBS/tween) was prepared. 50 μl of the diluted serum samples and the blank was dispensed in duplicate to the plate. The plate

Table 1. Experimental design.

Mouse strains	Groups	Doses (mg/kg b. w.)	Week 0	Week 4	Week 6
BALB/c	PZQ 450	450	I	T	S(6) P(6)
	PZQ 900	900	I	T	S(6) P(6)
	PZQ 1350	1350	I	T	S(6) P(6)
	IC	-	I	S(6)	S(6) P(6)
Swiss	PZQ 450	450	I	T	S(6) P(6)
	PZQ 900	900	I	T	S(6) P(6)
	PZQ 1350	1350	I	T	S(6) P(6)
	IC	-	I	S(6)	S(6) P(6)

Exp, Experimental; **IC,** infected control; **I,** infection; **T,** treatment; **S,** sampling; **P,** perfusion; **mg/kg b. w.,** milligram/kilogram body weight; no activity, ( )-Number of mice per group.

was incubated for 2 h at 37°C to allow any antibodies present to bind to the antigens after which the plates were washed six times. 50 µl of the conjugate, goat anti-mouse immunoglobulin G horse radish peroxidase (HRP) diluted 1:2000 was added in each well. The plate was incubated for 1 h at 37°C to allow binding of the secondary antibody to the primary one and washed six times. 50 µl of the substrate (TMB microwell peroxide substrate Kirkguard and Perring laboratories, USA) was added and the plate incubated in the dark for 30 min. Colour developed depending on the strength of binding. The plates were read at 630 nm on an ELISA reader, Marxi kinetic microplate reader (Molecular Devices, Palo Alto, England).

Perfusion and worm recovery

Based on modified method of Smithers and Terry 1965, mice were anaesthetized and hepatic portal vein incised. Perfusion needle containing perfusion fluid (0.85% sodium chloride and 15% sodium citrate) was inserted on the left ventricle of the heart and perfusion carried out until the liver, lower limbs and mesenteric were clear. The perfusate was collected in glass Petri dish (20 cm diameter) and transferred in a urine jar to settle. The adult worms were recovered using the method described by Yole et al. (1996); urine jar with perfusate containing the recovered worms was topped with phosphate buffered saline (PBS). After the worms settled, the supernatant was sucked out, and the settling procedure repeated three times. When the supernatant was clear, the worms were poured on a Petri dish containing PBS and counted. The worm maturation, percentage worm recovery and percentage worm reduction of adult worm recovered for each group was calculated.

Gross and histopathological assessment

Just before initiation of perfusion, gross pathology was carried out on liver tissues. Macroscopical observation was made by comparing tissues from the infected control and experimental groups with those of normal naïve mice. Liver was assessed in terms of inflammation, adhesions and granulomas. After perfusion, the livers were fixed in 10% buffered formalin for at least 2 weeks. Tissues were cleared in toluene, infiltrated in hot paraffin and embedded on tissue-embedding paraffin wax. Tissues were sectioned serially at 6 microtomes, using a rotary microtome. The thin tissue sections were mounted on glass slides and stained with haematoxylin and eosin. The tissue sections were observed under light microscope for any pathological changes. Liver pathology was observed in terms of granuloma sizes, immune cells infiltration and fibrosis (Baker et al., 1989).

Data analysis

Analysis of the data was performed using SPSS student's t-test for comparison of two paired samples e.g. comparison of one treatment and control. Significance was defined as p-value of < 0.05.

RESULTS

Worm maturation in BALB/c and Swiss mice Worm maturation from infected control (IC) mice is calculated as follows:

$$\text{Worm maturation} = \frac{\text{Number of worms recovered from infected control}}{\text{Initial number of infecting cercariae}} \times 100$$

Worm maturation in infected control BALB/c was 10%, while that in Swiss mice was 14%. Swiss mice had more infecting parasites maturing into adult worms than BALB/c mice.

Worm recovery in BALB/c and Swiss mouse strains

Adult male and female *S. mansoni* worms were recovered from the groups of mice that were challenged with 250 cercariae. The mean number of *S. mansoni* worms that were recovered from BALB/c mice in PZQ 1350 (3.06 ± 0.30), PZQ 900 (8.17 ± 1.34), PZQ 450(11.33 ± 3.39) and IC (24.571 ± 3.07) are shown in Table 1. The percentage worm recoveries from BALB/c mice PZQ1350 (30.30%), PZQ 900 (33.24%) and PZQ 450 (46.12%) are shown in Figure 1. IC was statistically
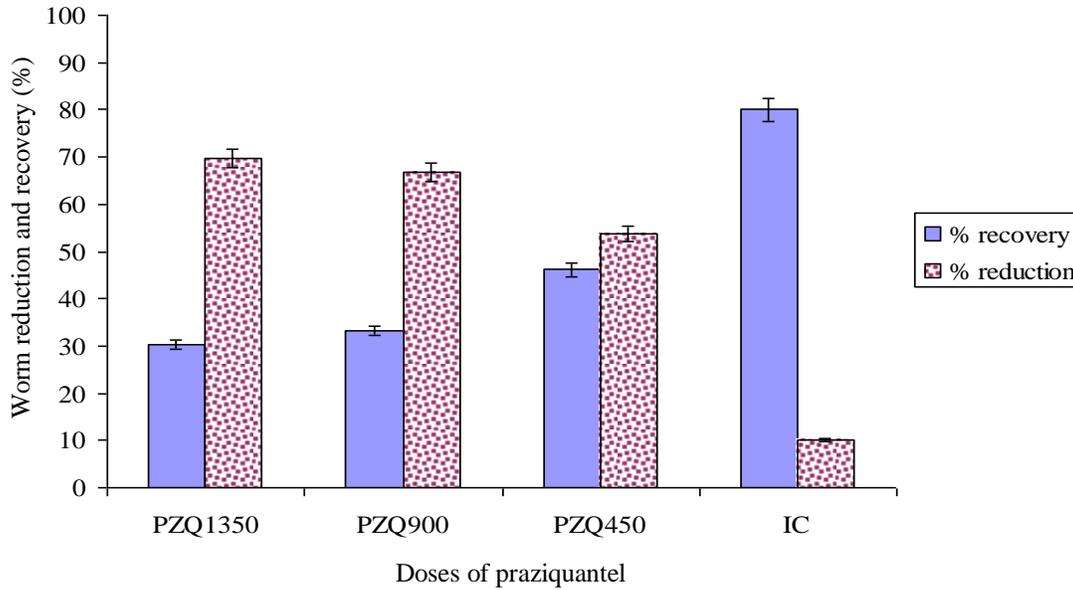
Figure 1. Percentage Schistosome worm recovery and worm reduction in BALB/c mouse strain in different treatment. PZQ 1350 – Mice receiving 1350 mg/kg b. w. of PZQ; PZQ900 – mice receiving 900 mg/kg b.w.of PZQ; PZQ 450 – Mice receiving 450mg/kg b.w. of PZQ; IC6 – mice infected only.

different from all other groups when pair wise comparison was performed. There were significant differences between IC and PZQ 1350 (P < 0.001), IC and PZQ 450 (P < 0.001) and IC and PZQ 900 (P < 0.001). PZQ 1350 dose exhibited statistical difference when compared with PZQ 450 (P < 0.045). There was significant difference between PZQ 1350 and PZQ 900 dosages (P < 0.001). PZQ 1350 had the lowest worm recovery (3.06 ± 0.30) followed by PZQ 900 (8.17 ± 1.34). PZQ 450 dosage had higher worm recovery (11.33 ± 3.39) compared with both PZQ 1350 and PZQ 900 dosages, however IC had the highest worm recovery compared with all dosages as shown in Table 1. Adult male and female *S. mansoni* worms were recovered from the groups of mice that were challenged with 250 cercariae. The percentage worm recoveries from Swiss mice PZQ 1350 (34.08%), PZQ 900 (47.52%) and PZQ 450 (75.66%) are shown in Figure 2. IC was statistically different from all other groups when pair wise comparison was performed. There were significant differences between IC and PZQ 1350 (P < 0.001). PZQ 900 dosage showed significant difference

with IC (P < 0.001), while there were no statistical differences between IC and PZQ 450 (P > 0.05). PZQ 1350 dose exhibited statistical difference when compared with PZQ 450 (P < 0.001). PZQ 450 was significantly different when compared with PZQ 900 (P < 0.001). There were significant differences between PZQ 1350 and PZQ 900 dosages (P < 0.001). PZQ 1350 had the lowest worm recovery (3.06 ± 0.30) followed by PZQ 900 (8.17 ± 1.34). PZQ 450 dosage had higher worm recovery (11.33 ± 3.39) compared with both PZQ 1350 and PZQ 900 dosages; however, IC had the highest worm recovery compared with all dosages as shown in Table 2.

Percentage worm recovery and percentage worm reduction in BALB/c and Swiss mice

Percentage worm reduction and worm reduction were calculated as follows:

$$\% \text{ worm recovery} = \frac{\text{Mean of total worms in experimental group}}{\text{Mean of total worms in infected control}} \times 100$$

$$\% \text{ worm reduction} = \frac{\text{Mean of total worms in infected control} - \text{Mean of total worms in experimental group}}{\text{Mean of total worms in infected control}} \times 100$$

The percentage schistosome worm reduction from BALB/c mice in groups PZQ 1350, PZQ 900 and PZQ

450 are shown in Figure 1. PZQ 1350 had the highest worm reduction (69.70%) followed by PZQ 900 (66.76%)

Table 2. Gross pathology in BALB/c and Swiss mice.

Dosage group	Adhesions		Inflammation		Granuloma	
	BALB/c mice	Swiss mice	BALB/c mice	Swiss mice	BALB/c mice	Swiss mice
PZQ 1350						
M	√	√	√ S	√ S		
M	√	√	√ S	√ S		
M	√	√	√ S	√ S		
F	√	√	√ S	√ S		
F	√	√	√ S	√ S		
F		√	√ S	√ S		
PZQ 900						
M	√	√	√ S	√ S		
M		√	√ S	√ S		
M		√	√ S	√ S		
F	√	√	√ S	√ S		
F		√	√ S	√ S		
F		√	√ S	√ S		
PZQ 450						
M	√	√	√ S	√ S	√ FG	√ FG
M	√	√	√ S	√ S		
M	√	√	√ S	√ S		
F	√	√	√ S	√ S		
F	√	√	√ S	√ S		
F	√	√	√ S	√ S		
IC						
M	√	√	√ *	√ S		√ FG
M	√	√	√ *	√ S		
M	√	√	√ *	√ S		
F	√	√	√ *	√ S	√ MG	√ FG
F	√	√	√ *	√ S	√ MG	√ FG
F	√	√	√ *	√ S		

* - Very inflamed, S - slightly inflamed, FG - few granuloma, MG - moderate GRANULOMA, √- pathology, M - male, F – female.

and PZQ 450 (53.88%) had the lowest worm reduction.

The percentage schistosome worm reduction from Swiss mice in groups PZQ 1350, PZQ 900 and PZQ 450 are shown in Figure 2. PZQ 1350 had the highest worm reduction (65.92%) followed by PZQ 900 (52.48%), and PZQ 450 (24.34%) had the lowest worm reduction.

Antibody responses to schistosome antigens

Soluble schistosomule protein (SSP) - specific immunoglobin (IgG) responses in BALB/c mouse strain

The percentage schistosome worm reduction from Swiss IgG responses for SSP were measured in serum samples collected from BALB/c mice using enzyme linked immunosorbent assay (ELISA); the results are shown in Figure 3. PZQ 1350 (0.787 ± 0.02) had the highest IgG response to SSP compared to all treatments followed by PZQ 900 (0.315 ± 0.04) and PZQ 450 (0.298 ± 0.01). Among the controls, IC6 (0.541 ± 0.05) had the highest IgG response followed by naïve (0.211 ± 0.02) and the least response in IC4 (0.165 ± 0.05). PZQ 1350 showed significant difference when compared with PZQ 900 (p < 0.001), and PZQ 450 (p < 0.001). There was no significant difference between PZQ 900 and PZQ 450 (p > 0.05). IC6 exhibited significant difference when compared with PZQ 1350 (p < 0.001), PZQ 900 (p < 0.001) and PZQ 450 (p < 0.001) dosages. IC6 was significantly different when compared with IC4 (p < 0.05).

Schistosome worm antigen preparation (SWAP) - specific IgG responses in BALB/c mouse strain

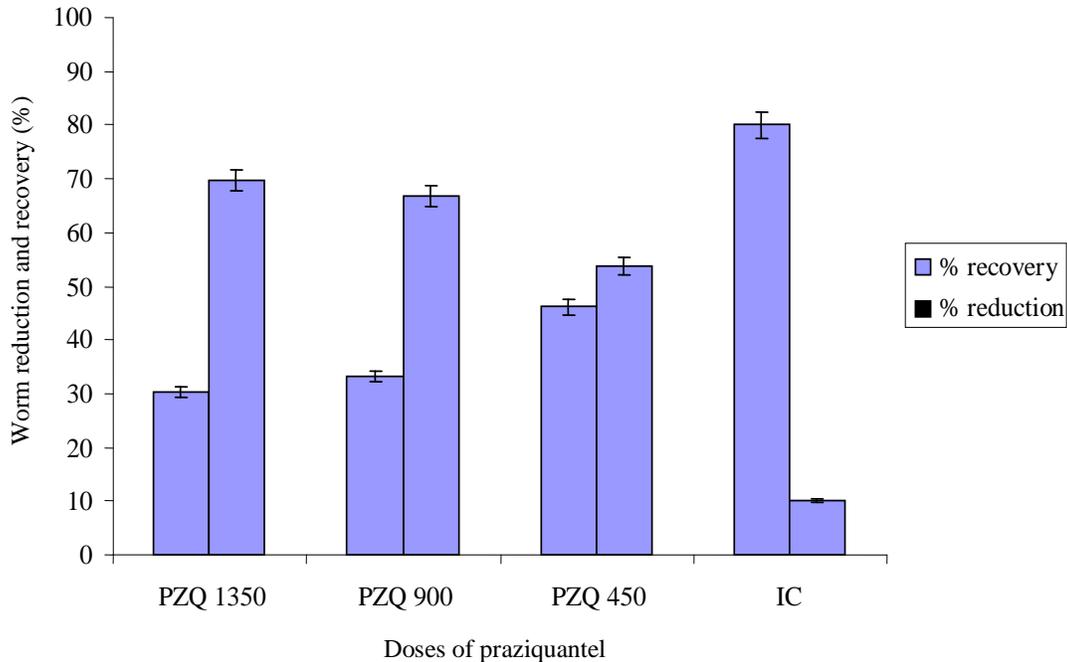IgG levels for SWAP as shown in Figure 4. PZQ 1350

Figure 2. Percentage Schistosome worm recovery and worm reduction in swiss mouse strain in differenttreatment. PZQ 1350 – Mice receiving 1350 mg/kg b. w. of PZQ; PZQ900 – mice receiving 900 mg/kg b.w.of PZQ; PZQ 450 – Mice receiving 450mg/kg b.w. of PZQ; IC6 – mice infected only.

(0.891 ± 0.02) had the highest immunological response compared to all treatments, followed by PZQ 450 (0.742 ± 0.06) and PZQ 900 (0.700 ± 0.04). Among the controls IC6 (0.469 ± 0.04) had the highest IgG response followed by IC4 (0.172 ± 0.06) as shown in Figure 4. PZQ 1350 was statistically different when compared with PZQ 900 and PZQ 450 (P < 0.05). There were no differences statistically between PZQ 900 and PZQ 450 (P > 0.05). IC6 showed significant differences when compared with PZQ 1350 (P < 0.001), PZQ 900 (P<0.01) and PZQ 450 (P<0.05). IC6 recorded significant differences when compared with IC4 (P < 0.05).

SWAP - specific IgG responses in Swiss mouse strain

IgG levels for SWAP for Swiss mice are shown in Figure 4. PZQ 1350 (0.785 ± 0.02) had the highest IgG response among the treatments followed by PZQ 450 (0.659 ± 0.02) and PZQ 900 (0.642 ± 0.01). Among the controls, IC6 (0.533 ± 0.03) had the highest IgG response followed by IC4 (0.419 ± 0.01) as shown in Figure 4. PZQ 1350 dose was significantly different when compared with PZQ 900 (P < 0.001), and PZQ 450 (P < 0.05). There was no significant difference between PZQ 900 and PZQ 450 (P > 0.05). IC6 had significant differences when compared with PZQ 1350 (P < 0.001), PZQ 900 (P < 0.05) and PZQ 450 (P<0.05). IC6 showed significant difference compared with IC4 (P < 0.05).

SSP – specific IgG responses in Swiss mouse strain

IgG levels for SSP are shown in Figure 5. PZQ 1350 (0.825 ± 0.02) had the highest immunological response among the treatments followed by PZQ 450 (0.314 ± 0.02) and PZQ 900 (0.296 ± 0.02). Among the controls, IC6 (0.203 ± 0.04) had the highest IgG response followed by IC4 (0.446 ± 0.05) (Figure 4). PZQ 1350 was statistically higher than PZQ 900 (p < 0.001), and PZQ 450 (p < 0.001). There was no significant difference between PZQ 900 and PZQ 450 (p > 0.05). IC6 was significantly different when compared with PZQ 1350 (p < 0.001), PZQ 900 (p < 0.05) and PZQ 450 (p < 0.05). IC6 was significantly higher than IC4 (p < 0.01).

Pathological findings in BALB/c and Swiss mouse strains

Gross pathology observations were done on week 6 post infection by physical observation of the liver to detect presence of granulomas, inflammation and adhesions. The results are shown in Table 4. There were adhesions in each of the six mice tested in all groups of BALB/c mice except PZQ 900 had adhesions only in two mice (Table 4). There was slight inflammation in the livers of all the groups, except IC in which livers of all mice were very inflamed. PZQ 1350 and PZQ 900 had no granulomas while PZQ 450 had only one male with few granulomas. IC had two mice, which recorded moderate granulomas.
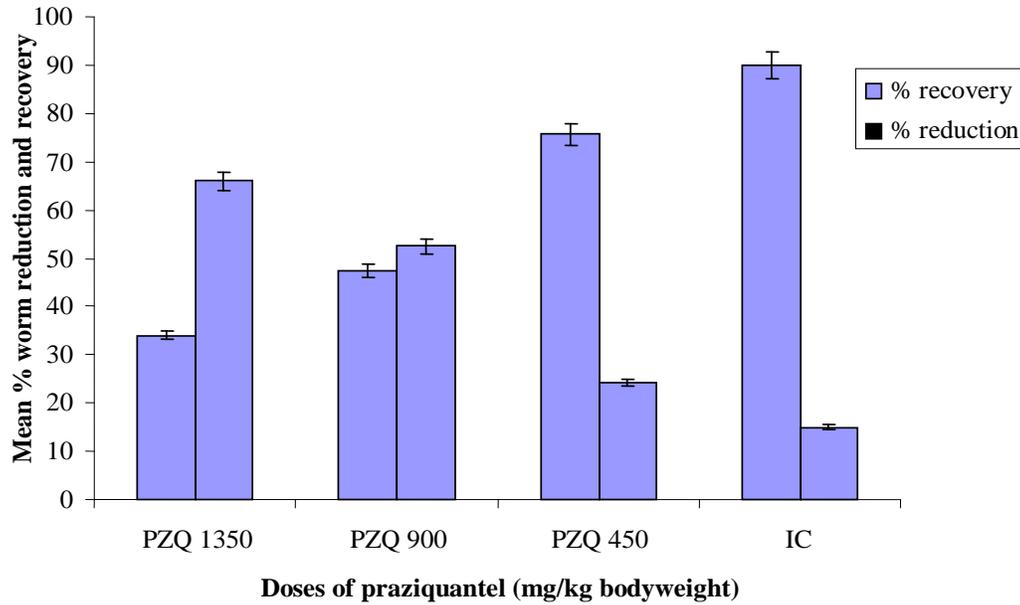
Figure 3. SSP and SWAP specific IgG antibody levels in group PZQ 1350, PZQ 900, PZQ 450, IC6, and IC4 in BALB/c mice strain. PZQ 1350 – mice receiving 1350 mg/kg b. w. of PZQ; PZQ 900 – Mice receiving 900 mg/kg b. w. of PZQ; PZQ 450 – Mice receiving 450 mg/kg b. w. of PZQ; IC6 – Mice infected only and serum obtained at week 6 after infection; IC4 – Mice infected only and serum obtained at week 4 after infection.
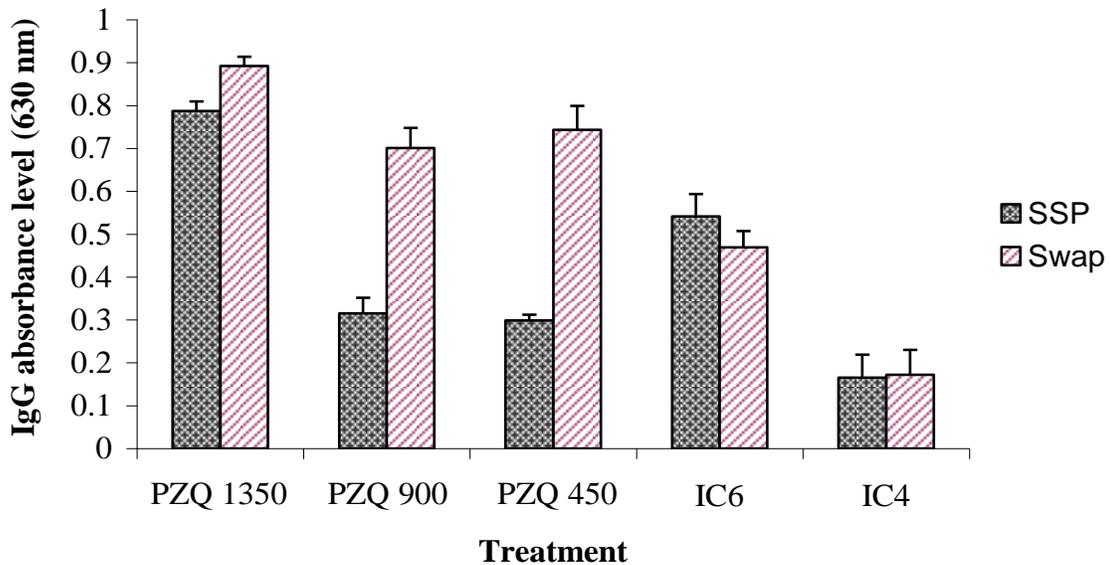

Figure 4. SSP and SWAP specific IgG antibody levels in group PZQ 1350, PZQ 900, PZQ 450, IC6, and IC4 in Swiss mice strain. PZQ 1350 – Mice receiving 1350 mg/kg b.w. of PZQ; PZQ900 – Mice receiving 900 mg/kg b. w. of PZQ; PZQ450 – Mice receiving 450 mg/kg b. w. of PZQ; IC6 – Mice infected only and serum obtained at week 6 after infection; IC4 – Mice infected only and serum obtained at week 4 after infection.

There were adhesions in each of six mice tested in all groups of Swiss mice (Table 4). All groups had slightly inflamed livers. PZQ 1350 and PZQ 900 had no granulomas while PZQ 450 had only one male with few granulomas. In IC, there were few granulomas in one male and two females.
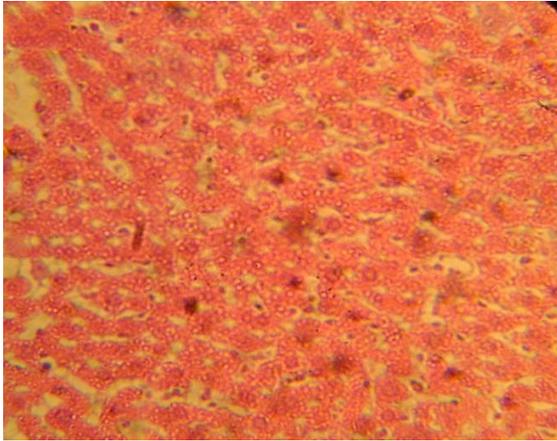
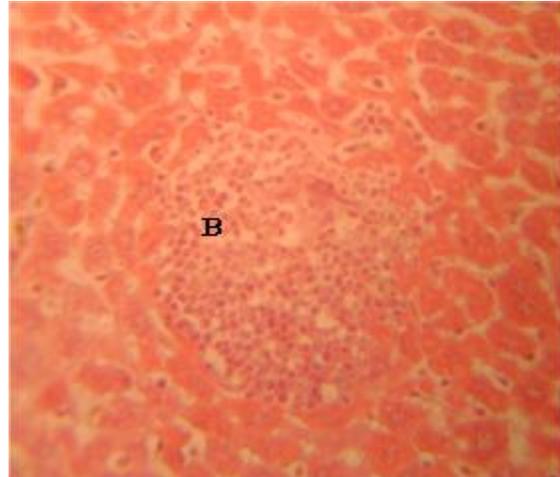**Figure 5.** Normal liver tissue (H&E, 100✕).

Figure 7. Hepatic periovular granuloma in a mouse at six weeks after treatment with PZQ 450 shows phagocytosed non viable schistosome egg (B) (H&E, 100✕).
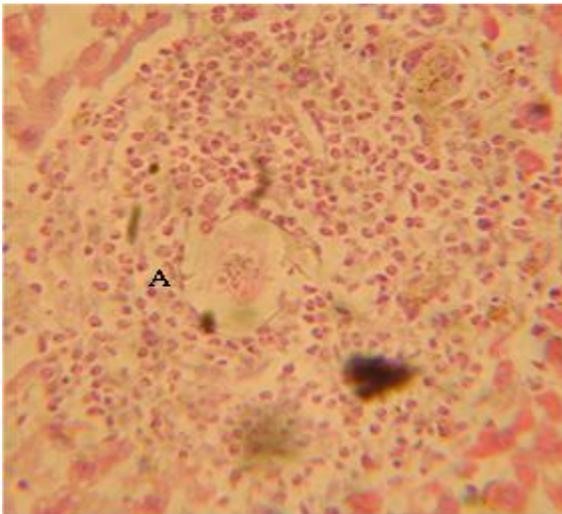

Figure 6. Hepatic periovular granuloma in a mouse at six weeks after infection shows a non viable schistosome egg (A) (H&E, 100✕).

Histopathology in BALB/c and Swiss mice strains

In both BALB/c and Swiss mice, normal liver tissue (Figure 5) was encountered in PZQ 1350 and PZQ 900. Granulomas with visible eggs (Figure 6) were encountered in PZQ 450 and more frequently in infected control. Also, these two groups had areas of cellular infiltration (Figure 7) as results of diffusing egg antigens were encountered.

DISCUSSION

Worm maturation in BALB/c and Swiss mice

The percentage number of worms that matured from

BALB/c infected control group was 10% lower compared to those that in matured Swiss mice infected control group which recorded 14%. Therefore, Swiss mouse was a better permissive host than BALB/C as it allowed more parasites to mature instead of destroying them. In the mouse model system, only about 20% of initial cercarialinoculum makes it to the adult stage. It is clear that the greatest loss of larval stages occurs during the migration through the lungs with relatively smaller losses during migration through the skin (WHO, 2002).

Worm recovery and worm reduction in BALB /c and Swiss mice strain

The mean number of worms recovered from the four groups showed an increasing trend from PZQ 1350, PZQ 900, PZQ 450, and infected control in Swiss mice and BALB /c mice. The significant low worm recovery and more worm reduction at PZQ 1350 were probably due to the action of the drug because most of the worms had been destroyed compared to other dosages. In PZQ 900, few worms were recovered and a lot reduced compared to PZQ 450, due to the effect of the dosage, whereas, more worms were recovered and less reduced at PZQ 450, since the dosage was not effective at clearing the worms. However, infected control group recorded high number of worms because most of the worms had matured and migrated to the mesenteries and were recovered during perfusion without any drug induced destruction. Therefore, increase in dosage decreased number of worms recovered. PZQ damages the adult worm tegument, releasing large amounts of antigens directly into the blood stream. Praziquantel causes irreversible focal vacuolization with subsequent cestodal

disintegration at specific sites of the integument (Ali, 2006).

In schistosomes and other trematodes, praziquantel directly kills the parasite, possibly by increasing calcium ion (Ca^{2+}) flux into the worm. There is experimental evidence that praziquantel increases the permeability of the membranes of parasite cells for Ca^{2+}.

The drug thereby induces contraction of the parasites resulting in paralysis in the contracted state. The dying parasites are dislodged from their site of action in the host organism and may enter systemic circulation or may be destroyed by host immune reaction. Additional mechanisms are focal disintegrations and disturbances of ovipositor (laying of eggs), as are seen in other types of sensitive parasites. Focal vacuolization of the integument follows and the parasite is phagocytozed. Praziquantel may adversely affect the parasite's glutathione and intracellular calcium concentrations, with secondary effects on the metabolism and antigenicity (Ali, 2006). It is therefore apparent that increasing dosage increases power to destroy worms.

Antibody responses to schistosome antigens in BALB/c and Swiss mice

Antibody responses recorded in *S. mansoni* SSP, specific IgG antibody levels were high in PZQ 1350 followed by PZQ 900; however, there was slight difference in response at PZQ 450 compared to PZQ 900. SSP specific IgG responses were high in group treated with PZQ 1350 due to stimulation of antibodies by antigens released from dead worms. There were also IgG specific responses in the other dosages, PZQ 900 and PZQ 450, though they were not very different which indicated that the higher the dosage, the more worm destruction that occurred and the higher the antibody response that resulted due to released antigens from dead worms. IC6 had high antibody response compared to IC4 because worms were mature at week 6 compared to week 4; hence, more antigens were being released stimulating increased antibody response at week 6.

The results therefore indicated that PZQ1350 was a better dosage. There was more SWAP IgG specific response at PZQ 1350 but there was slight difference in both PZQ 900 and PZQ 450; hence, the more the dosage, the more the synergy. IC6 recorded more IgG specific response compared to IC4. SSP specific IgG responses showed a decreasing trend in Swiss mice groups treated with PZQ 1350 followed by PZQ 900 and PZQ 450. The last two did not show much difference; this showed that the higher the dosage the more worms that were destroyed. Hence PZQ 1350 had more worm reduction and high antibody response. This was then followed by PZQ 900 and PZQ 450. There was difference in IC6 SSP specific IgG response compared to IC4, at this time more worm had matured to larval stage and

more antigens were being released thus provoking the humoral immune response through production of antibodies that participated in dependent cellular cytotoxicity *(ADCC)* damaging schistosomula stage (Hagan et al., 1998). Treatment with praziquantel damages the tegument of schistosome (Andrew, 1995), exposing worm antigens (Fallon and Doenhoff, 1995) to the immune system. This action induces both humoral and cellular responses resulting in the death of worm (Woolhouse and Hagan, 1999). Due to damage of schistosomes' regimen (Andrew, 1995) exposing worm antigens (Fallon and Doen, 1995) to the immune system, hence, there was more antibody response due to the synergistic action of the drug and the immune system.

It has also been shown that schistosomula stage is also vulnerable to host immunity (Terry, 1994; Ridi et al., 2001). From the result, PZQ 1350 was a better dosage in BALB/c mice compared to Swiss mice due to more antibody response compared to other dosages. Hence BALB/c was a better model compared to Swiss mouse strain. This leads to the activation of the immune system, thus killing the worms and mature eggs (Andrew, 1985; Richard et al., 1989). The action of praziquantel also exposes the worm surface antigens to the immune system, which induces both humoral and cellular responses that result in the killing of the worms (Wool and Hagan, 1999). In comparison, more worms were recovered and reduced in BALB/c than in Swiss mice.

Pathological findings

Granulomas are tiny pinhead sized foci on the surface of the liver that indicate cellular infiltration consisting mainly of eosinophils, macrophages, fibroblasts, and lymphocytes surrounding a schistosome tissue trapped egg (Hagan et al., 1998). To the naked eye, they appear as pin-sized white/cream spots giving the surface a rough texture. Though pathogenic, granulomas serve as an essential host protective function (Hagan et al., 1998). The color appears pale as opposed to red-pink in normal livers and the texture becomes rough rather than smooth. Granulomas were classified as none (0), few (1 to 3), moderate (4 to 10) and severe (>10) granuloma per liver lobe. Inflammation is a reaction of a tissue to infection due to cellular infiltration and it is characterized by redness, swelling, pain and heat. Adhesions are clear membranes that form in response to infection, and attach different tissues or parts of the same tissue.

Examination of livers for pathology at week 6 post infection showed all groups had adhesion in both BALB/c and Swiss mice except PZQ 900, where four mice did not record. In terms of inflammation, all groups of Swiss mice were slightly inflamed but BALB /c mice PZQ 1350, PZQ 900 and PZQ 450 recorded slight inflammation but IC was severely inflamed. Granulomas were absent in PZQ 1350 and PZQ 900 of both BALB/c and Swiss mice, but

PZQ 450 and IC recorded few and moderate granulomas. PZQ 1350 was a better dosage for reducing egg associated pathology followed by PZQ 900 and finally PZQ 450.

In terms of models, BALB/c recorded more pathology compared to Swiss mice. Microscopically, PZQ 1350 and PZQ 900 recorded a normal liver tissue which indicated that the two treatments were able to destroy Schistosoma egg associated pathology. While, PZQ 450 had granulomas surrounding dead worms indicating that the dosage was not effective in destroying the worms.

Conclusion

The results of the study showed that PZQ 1350 had the greatest effect on worm reduction, worm recovery, pathology on liver tissue and IgG specific immunological response compared to other groups. This was followed by PZQ 900 which had lower number of worms reduced, worm recovery, absence of pathology and IgG specific immunological response compared with PZQ 1350. However, PZQ 450 had the least number of worm reduction, highest worm recovery, more pathology and least IgG specific immunological response as compared to PZQ 1350. This revealed that the increase in dosage decreases the number of worms recovered, increases worm reduction, reduces pathology and enhances synergy enabling destruction of worms by immune responses. The results showed that Swiss is a better model compared to Swiss mice since it is able to allow more worm maturation and worm recovery.

ACKNOWLEDGEMENTS

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