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# Combination therapy using Pentostam and Praziquantel improves lesion healing and parasite resolution in BALB/c mice co-infected with *Leishmania major* and *Schistosoma mansoni*

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## Abstract

**Background:** Most natural host populations are exposed to a diversity of parasite communities and co-infection of hosts by multiple parasites is commonplace across a diverse range of systems. Co-infection with *Leishmania major* and *Schistosoma mansoni* may have important consequences for disease development, severity and transmission dynamics. Pentavalent antimonials and Praziquantel (PZQ) have been relied upon as a first line of treatment for *Leishmania* and *Schistosoma* infections respectively. However, it is not clear how combined therapy with the standard drugs will affect the host and parasite burden in concomitance. The aim of the current study was to determine the efficacy of combined chemotherapy using Pentostam and PZQ in BALB/c mice co-infected with *L. major* and *S. mansoni*.

**Methods:** The study used BALB/c mice infected with *L. major* and *S. mansoni*. A 3 × 4 factorial design with three parasite infection groups (Lm, Sm, Lm + Sm designated as groups infected with *L. major*, *S. mansoni* and *L. major* + *S. mansoni*, respectively) and four treatment regimens [P, PZQ, P + PZQ and PBS designating Pentostam<sup>®</sup> (GlaxoSmithKline UK), Praziquantel (Biltricide<sup>®</sup>, Bayer Ag. Leverkusen, Germany), Pentostam + Praziquantel and Phosphate buffered saline] as factors was applied. In each treatment group, there were 10 mice. Lesion development was monitored for 10 weeks. The parasite load, body weight, weight of the spleen and liver were determined between week 8 and week 10.

**Results:** Chemotherapy using the first line of treatment for *L. major* and *S. mansoni* reduced the lesion size and parasite loads but did not affect the growth response, spleen and liver. In the co-infected BALB/c mice, the use of Pentostam or PZQ did not result in any appreciable disease management. However, treatment with P + PZQ resulted in significantly ( $p < 0.05$ ) larger reduction of lesions, net increase in the body weight, no changes in the spleen and liver weight and reduced Leishman-Donovan Units (LDU) and worm counts than BALB/c mice treated with Pentostam or PZQ alone.

**Conclusions:** The present study demonstrated that the combined first line of treatment is a more effective strategy in managing co-infection of *L. major* and *S. mansoni* in BALB/c mice.

**Keywords:** Schistosomiasis, Leishmaniasis, *Leishmania major*, *Schistosoma mansoni*, Praziquantel, Drug efficacy, Pentostam, Co-infection, Combination therapy

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## Background

Most natural host populations are exposed to a diverse community of parasites, and with respect to epidemiologic overlaps and human economic dynamics, comorbidity of hosts by multiple parasites occurs across a diverse range of systems [1-4]. Recent studies in vertebrates suggest that interactions between co-infecting parasites have important consequences for disease development, severity and transmission dynamics [5,6]. Thus understanding the consequences of co-infections in a host may be crucial for a deeper understanding of the disease pathognomonic, epidemiology and effective disease control strategies.

In the developing world, leishmaniasis, caused by obligate intracellular kinetoplastid protozoa of the genus *Leishmania*, is endemic [7,8] and schistosomiasis, caused by parasitic trematodes (schistosomes) have widely been reported [9-13]. *Leishmania* and *Schistosoma* overlap extensively in their epidemiological distributions and occasionally co-infect the same individuals [14-21]. How these two types of parasite might interact within co-infected hosts and the associated epidemiology continues to be debated. One line of argument indicates that the interactions between the helminth and protozoan parasites could affect both parties [22], while others argue that the helminth/protozoan co-infection influences leishmaniasis development without any effect on the helminth parasite [23-25]. This bias may reflect the greater human disease burden imposed by leishmaniasis compared to helminths [26], as well as the ongoing need to understand what causes variability in leishmaniasis infection outcomes.

Interactions among parasitic agents commonly alter disease severity [1,27,28]. Co-infecting parasites may interact either positively (facilitation) or negatively (competition) via a range of mechanisms including resource competition, immune-mediated interactions and direct interference. To date, studies of helminth-protozoa co-infection have focused largely on immune-mediated mechanisms, no doubt largely due to the known immunomodulatory effects of helminths. Using the immune response mechanism, two major pathways have been proposed by which helminths might release parasites from immune pressure and thereby facilitate their replication, both of which involve the dampening of pro-inflammatory immune responses [29-34]. Thus it has been suggested that by polarizing immune responses towards Th2-type effector mechanisms, helminths will diminish the pro-inflammatory Th1-type mechanisms needed to down modulate *Leishmania* in comorbidity. These suggest that helminth co-infection might thus impair the mechanisms necessary to control leishmaniasis. The current immunomodulatory explanation is in concurrence with observations in murine models, where co-infection of *L. major* and *S. mansoni* exacerbated lesion development compared to mice infected with *L. major* alone [22-24,35]. The action of helminth infection affecting

the immune response of the host, may increase protozoan multiplication substantially thus enhancing leishmaniasis severity [23,24,26].

The chemotherapy of leishmaniasis is based on the use of antimonials, among which the most used are meglumine antimoniate (Glucantime®) and sodium stibogluconate (Pentostam®) [36-38]. The WHO recommended schistosomiasis control strategies for humans by focusing on the large-scale population-based and repeated chemotherapy, which is still the key strategy today. Three drugs have been used, which differ in their effects on schistosome species: metrifonate (targeting *S. haematobium*), oxamniquine (targeting *S. mansoni*), and PZQ (for all human species) [39,40]. Due to its broader spectrum, PZQ has finally become the first-line medicine. Yet, advances in antiparasitic chemotherapy have made combination chemotherapy a real possibility [41,42]. Drug combinations aim to delay or prevent the emergence of resistance, shorten the course of treatment and lower required doses. Other potential advances include convenience, better compliance and lower costs [43]. Nevertheless, the co-infection may potentially affect the chemotherapy outcome using standard and/or recommended drugs for single infections in the mice. As yet, the pathognomonic and subsequent treatment of protozoa and helminth concomitance in murine models is not well understood. The aim of the current study was to determine the efficacy of combined chemotherapy using Pentostam® and Praziquantel in BALB/c mice co-infected with *L. major* and *S. mansoni*. We hypothesize that treatment of BALB/c mice co-infected with *L. major* and *S. mansoni* using a combination of Pentostam and PZQ as the first line treatments for *L. major* and *S. mansoni* respectively may result in synergistic drug actions against the infection.

## Methods

### Experimental setup

The study applied a 3 × 4 factorial design with three parasite infection groups (Lm, Sm and Lm + Sm designated as groups infected with *L. major*, *S. mansoni* and *L. major* + *S. mansoni* respectively) and four treatment regimens [P, PZQ, P + PZQ and PBS designating Pentostam® (GlaxoSmithKline, UK), Praziquantel (Biltricide®, Bayer Ag. Leverkusen, Germany), Pentostam + Praziquantel and Phosphate buffered saline] as factors. In each treatment group, there were 10 mice. All treatments were executed in triplicate.

### Mice, parasites and experimental infections

Female (6–8 week old) BALB/c mice weighing 20 ± 2 g were used in the experiment. The animals were obtained from Kenya Medical Research Institute (KEMRI) animal breeding facility, Nairobi-Kenya. The animals were moved into the experimental room for acclimatization one week

before the start of the experiments. The mice were housed in 15 cm × 21 cm × 29 cm transparent plastic cages. They were fed with pellets (Mice pellets UNGA® feeds) and water *ad libitum*.

Kenya laboratory maintained (KEN-lab) strain of *S. mansoni* parasite was used in the study. The isolate had been routinely maintained in the laboratory at KEMRI by passage in *Biomphalaria pfeifferi* snails and inbred laboratory mice. For mouse infections, cercariae were obtained from infected snails, counted and applied percutaneously by the ring method [44] to mice anesthetized intraperitoneally with Pentobarbital sodium 80 mg/kg (Rompun; Bayer Plc., Newbury, UK) [45]. For the *S. mansoni* infection, mice were infected with 70 cercariae.

*L. major* (strain IDUB/KE/94 = NLB-144) was maintained by serial passage in BALB/c mice to maintain virulence. An aspirate isolate from the footpad of an infected BALB/c mouse was cultivated in Schneider's *Drosophila* insect medium (Sigma, Saint Louis, USA), supplemented with 20% heat inactivated foetal bovine serum (FBS) (Cultilab, Campinas, SP, Brazil), 500 µg/ml penicillin, 500 µg/ml streptomycin and 250 µg/ml 5-fluorocytosine arabinoside (all from Gibco, Grand Island, NY, USA) [46,47]. Promastigotes were incubated at 25°C and grown to stationary phase to generate infective metacyclic forms at 6th day of culture. Promastigotes in the medium were counted with a hemocytometer (Improved Double Neubauer) (Pharmacia-GE Healthcare, Uppsala, Sweden) with a Nikon optiphot optical microscope at 40× magnification. Mice were inoculated with 1 × 10<sup>6</sup> stationary phase *L. major* promastigotes in 50 µl PBS into the Left Hind Footpad (LHFP) using a 29 gauge needle.

#### Treatment

At the 5th week post infection (day 29), each of the parasite infection groups, were either treated with Pentostam (P), Praziquantel (PZQ), P + PZQ or PBS for the controls. 20 mg/kg bodyweight/day standard dose of Pentostam (GlaxoSmithKline, UK), was prepared and administered intraperitoneally (ip) for 28 days [48]. Praziquantel (Biltricide®, Bayer Ag. Leverkusen, Germany) was suspended in PBS and was administered to the mice by gavage at a total dosage of 600 mg/kg body weight (divided into 2 equal doses of 300 mg/kg) given 8 hours apart [49,50].

#### Sampling, lesion measurement and determination of parasite burden

A total of five mice were sampled at week 8 and week 10 for analysis of *L. major* parasite loads and *S. mansoni* worm counts. Lesion sizes of *L. major* infected mice, which was defined as the difference in thickness between the infected footpad and the non-infected contralateral footpad, was monitored weekly by measurement

using a Starret dial caliper (Mitutoyo, Suzano, SP, Brazil) [24,51]. The weight of the mice was also monitored on a weekly basis.

*S. mansoni* worms were recovered by portal perfusion [52], with perfusion buffer [phosphate buffered saline, 0.02 µl/ml heparin (monoparin; CP Pharmaceuticals Ltd., Wrexham, UK)]. The worms were washed free of erythrocytes and counted using a dissecting microscope. The percentage worm recovery and percentage worm reduction was calculated [50] as shown below:

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

$$\% \text{worm reduction} = \frac{\text{Mean of worms in Infected Control} - \text{Mean of worms in Experimental group}}{\text{Mean of worms in infected control}} * 100$$

The liver and spleen were removed and weighed and changes post-infection and chemotherapy were determined based on percentage reduction/increase, spleno-somatic and hepato-somatic indices [50]. Splenic *L. major* burdens were determined from Giemsa-stained impression smears and expressed as Leishman-Donovan units (the number of amastigotes per 1000 host nuclei, multiplied by the weight of the organ) [53-55].

#### Ethical clearance

All procedures were approved by the ethics committees for animal care and research: KEMRI Animal Care and Use Committee (ACUC), Scientific Steering Committee (SSC) and Ethical Review Committee (ERC). The guidelines were strictly adhered to during the research.

#### Statistical analysis

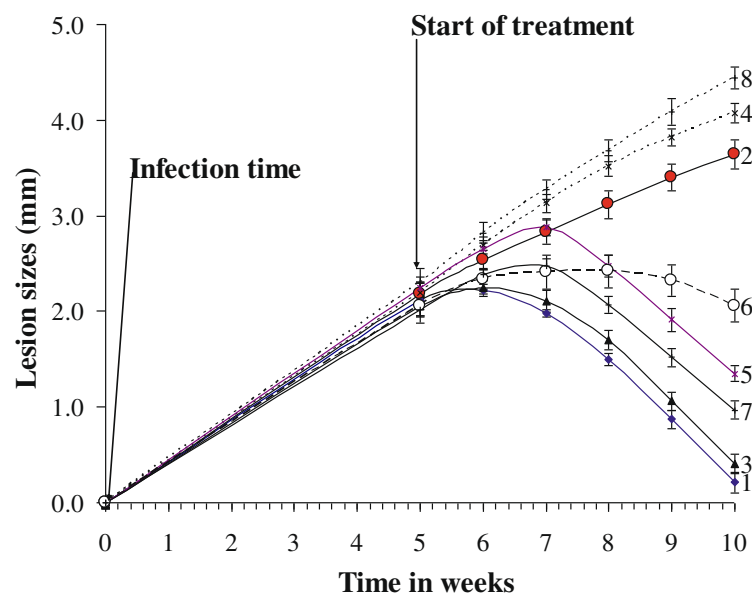
All statistical analyses were performed with a version of STATISTICA 10.0 statistical packages. Normality of data distributions were checked by means of the kurtosis to determine any need for applying appropriate data transformation procedures [55]. For each parameter analyzed, differences among treatment groups exposed to different drugs were tested by Factorial ANOVA. Comparison of efficacies among different treatment groups and time was carried out using factorial repeated measure ANOVA. Differences on *L. major* parasites and *S. mansoni* worm counts were analyzed using Friedman's Two-Way ANOVA as count data take discreet variables. Percentage data were arcsine transformed before subjecting the data to statistical analysis of Variance. In cases where significant differences were discerned means were compared using Tukey's HSD test [56]. All analyzed results were declared significant at  $p < 0.05$ .

## Results

The lesion sizes of BALB/c mice at the start of infection, at treatment and response after treatment with Pentostam, PZQ and combination of P + PZQ between week 8 and 10 is shown in Figure 1. During the first five weeks, there was a progressive increase of lesion size in BALB/c mice indicating progression of disease infection. There were significant differences in lesion sizes among different treatments ( $F = 21.1223$ ,  $p = 0.0001$ ) between week 5 to week 10. The largest reduction in lesion size after five weeks post infection followed by chemotherapy was observed in *L. major* infected BALB/c mice treated with Pentostam. Nevertheless, there was no ( $p > 0.05$ ) difference in lesion size of *L. major* infected BALB/c mice treated with either Pentostam or with a combination of P + PZQ. In the co-infected BALB/c mice, treatment with P + PZQ resulted in significantly ( $p < 0.05$ ) the largest reduction of lesion when compared with BALB/c mice treated with Pentostam or PZQ alone. PZQ had no effect on *L. major* infected BALB/c mice.

Body weights, weights of spleen and weights of the liver of BALB/c mice infected with *L. major*, *S. mansoni* and those co-infected with *L. major* + *S. mansoni* are

presented in Table 1. There were significant differences in the body weights, weight of spleen and in the weight of liver of BALB/c mice under different treatments between the eighth and tenth week ( $p < 0.05$ ). The largest increase in body weight occurred in mice infected with *L. major* and treated with Pentostam, in *S. mansoni* infected mice and treated with PZQ; nonetheless, combined therapy resulted in net increase in body weights of BALB/c mice singly infected with either *L. major* and *S. mansoni*. Treatment of *L. major* and *S. mansoni* co-infected BALB/c mice through monotherapy (either Pentostam or PZQ) resulted in reduced body weights while using combination therapy of P + PZQ resulted in a net increase in the body weight between week 8 and 10. It was observed that the largest increase in the weight of the spleen was in control groups. As for the infected and treated mice, in the *L. major* or *S. mansoni* infected mice, treatment with Pentostam and PZQ respectively, resulted in a slight and non-significant ( $p > 0.05$ ) increase in the weight of spleen between weeks 8 and 10. Simultaneous chemotherapy for either infection did not show any appreciable change in the weight of spleen. However, the largest increase in spleen weight occurred in BALB/c



### Legend

Treatment	Infection	Drug administration
1	<i>L. major</i>	P
2	<i>L. major</i>	PZQ
3	<i>L. major</i>	P+PZQ
4	<i>L. major</i>	PBS
5	<i>L. major</i> + <i>S. mansoni</i>	P
6	<i>L. major</i> + <i>S. mansoni</i>	PZQ
7	<i>L. major</i> + <i>S. mansoni</i>	P+PZQ
8	<i>L. major</i> + <i>S. mansoni</i>	PBS

**Figure 1** Lesion sizes of BALB/c mice at the start of infection, during the start of treatment with Pentostam, PZQ and combination of Pentostam and PZQ for 10 weeks.

**Table 1 Mean ( $\pm$  SEM) body weight, weight of spleen and weight of the liver of BALB/c mice infected with *L. major*, *S. mansoni* and those co-infected with *L. major* + *S. mansoni***

Parasite infection	Treatments	Body weights (g)			Weight of spleen (g)			Weight of the liver (g)		
		Week 8	Week 10	% change	Week 8	Week 10	% change	Week 8	Week 10	% change
<i>L. major</i>	P	18.2 $\pm$ 0.8	20.4 $\pm$ 1.4	12.1	0.14 $\pm$ 0.007	0.15 $\pm$ 0.012	7.14	0.81 $\pm$ 0.091	0.80 $\pm$ 0.084	-1.26
	PZQ	18.0 $\pm$ 0.5	16.6 $\pm$ 1.4	-7.77	0.20 $\pm$ 0.027	0.24 $\pm$ 0.009	20.00	0.84 $\pm$ 0.014	0.86 $\pm$ 0.025	2.13
	P + PZQ	18.6 $\pm$ 0.5	20.2 $\pm$ 1.2	8.60	0.15 $\pm$ 0.005	0.15 $\pm$ 0.007	0.00	0.79 $\pm$ 0.015	0.77 $\pm$ 0.014	-1.50
	PBS	18.4 $\pm$ 0.4	14.4 $\pm$ 1.1	-21.7	0.19 $\pm$ 0.027	0.24 $\pm$ 0.008	26.32	0.84 $\pm$ 0.011	0.86 $\pm$ 0.014	2.15
<i>S. mansoni</i>	P	18.0 $\pm$ 0.6	16.1 $\pm$ 1.1	-10.6	0.20 $\pm$ 0.010	0.24 $\pm$ 0.009	20.00	1.59 $\pm$ 0.023	1.64 $\pm$ 0.012	3.14
	PZQ	18.0 $\pm$ 0.6	19.8 $\pm$ 0.9	10.0	0.17 $\pm$ 0.022	0.19 $\pm$ 0.011	11.76	0.83 $\pm$ 0.006	0.82 $\pm$ 0.004	-1.26
	P + PZQ	18.6 $\pm$ 0.8	20.3 $\pm$ 0.6	9.10	0.16 $\pm$ 0.006	0.16 $\pm$ 0.005	0.00	0.83 $\pm$ 0.013	0.82 $\pm$ 0.049	-1.38
	PBS	18.4 $\pm$ 0.9	14.5 $\pm$ 1.7	-21.2	0.20 $\pm$ 0.025	0.25 $\pm$ 0.006	25.00	1.08 $\pm$ 0.011	1.11 $\pm$ 0.009	2.77
<i>L. major</i> + <i>S. mansoni</i>	P	19.8 $\pm$ 0.2	18.2 $\pm$ 0.5	-8.08	0.22 $\pm$ 0.006	0.25 $\pm$ 0.013	13.60	1.55 $\pm$ 0.041	1.58 $\pm$ 0.032	3.20
	PZQ	18.4 $\pm$ 0.3	17.0 $\pm$ 0.8	-7.60	0.22 $\pm$ 0.009	0.24 $\pm$ 0.007	9.09	1.59 $\pm$ 0.012	1.61 $\pm$ 0.011	1.95
	P + PZQ	18.4 $\pm$ 0.6	19.8 $\pm$ 0.4	7.60	0.19 $\pm$ 0.016	0.20 $\pm$ 0.012	5.26	0.82 $\pm$ 0.012	0.83 $\pm$ 0.013	1.22
	PBS	17.9 $\pm$ 0.9	14.4 $\pm$ 1.5	-19.6	0.21 $\pm$ 0.007	0.27 $\pm$ 0.018	28.57	1.04 $\pm$ 0.014	1.08 $\pm$ 0.017	4.24
ANOVA	<b>Main effect</b>									
	F	19.311	7.421		9.241	8.421		7.112	14.0214	
	<i>p</i> value	0.0000	0.0005		0.0001	0.0004		0.0071	0.0000	
	<b>Repeated measure</b>									
	F		9.2114			7.5515			5.5544	
	<i>p</i> value		0.0007			0.0021			0.0081	

infected with *L. major* and treated with PZQ, and in BALB/c infected with *S. mansoni* and treated with Pentostam. In the co-infected mice, treatment with Pentostam or PZQ alone resulted in a significant ( $p < 0.05$ ) increase in the weight of spleen between weeks 8 and 10. On the contrary, treatment of BALB/c co-infected with *L. major* + *S. mansoni* with P + PZQ resulted in slight but non-significant ( $p > 0.05$ ) increase in weight of the spleen. Treatment of BALB/c mice infected with *L. major* or *S. mansoni* with Pentostam and PZQ respectively or a combination of P + PZQ resulted in a slight reduction in the weight of the liver. There were opposing trends in the weight of the liver when treatment of *L. major* or *S. mansoni* infection was conducted using PZQ and Pentostam respectively. In mice, co-infected with *L. major* + *S. mansoni* there were significant ( $p < 0.05$ ) increases in the weight of the liver when Pentostam and PZQ were used as respective chemotherapeutants. Nevertheless, in co-infected mice, treatment with combined therapy of P + PZQ resulted in a slight but non-significant ( $p > 0.05$ ) increase in the liver weight. All the controls resulted in significant ( $p < 0.05$ ) increases in weight of the liver.

The spleno-somatic and hepato-somatic indices of BALB/c infected mice under different treatments are shown Table 2. The spleno-somatic index and hepato-somatic index of *L. major* infected BALB/c mice reduced

significantly ( $p < 0.05$ ) between week 8 and week 10 when treatment was carried out using Pentostam and P + PZQ but increased with treatment using PZQ. In BALB/c mice infected with *S. mansoni*, treatment with Pentostam resulted in a significant ( $p < 0.05$ ) increase of spleno-somatic and hepato-somatic indices. Meanwhile treatment of *S. mansoni* infected BALB/c mice with PZQ resulted in a non-significant ( $p > 0.05$ ) increase in the spleno-somatic index but significantly reduced the hepato-somatic index. Combined therapy of P + PZQ significantly ( $p < 0.05$ ) reduced the spleno-somatic and hepato-somatic indices. In co-infected BALB/c mice, treatment with either Pentostam or PZQ resulted in a significant ( $p < 0.05$ ) increase in both the spleno-somatic and hepato-somatic indices. However, when treatment was done using combined therapy of P + PZQ, the spleno-somatic index decreased slightly but with a larger significant ( $p < 0.05$ ) reduction observed in hepato-somatic index. Regardless of the parasitic levels of infection of the BALB/c mice, all mice maintained in the control groups had increased spleno-somatic and hepato-somatic index between week 8 and 10.

In *L. major* infected BALB/c mice, treatment with Pentostam and P + PZQ resulted in the lowest LDU, which significantly ( $p < 0.05$ ) reduced between week 8 and week 10 (Figure 2). In the PZQ treated and control groups, there was a significant increase in the LDU between week 8 and week 10. In BALB/c mice co-infected



**Table 2** The spleno-somatic and hepato-somatic indices of BALB/c infected with *L. major*, *S. mansoni* and those co-infected with *L. major* + *S. mansoni* undergoing various treatments with Pentostam and PZQ between week 8 and week 10

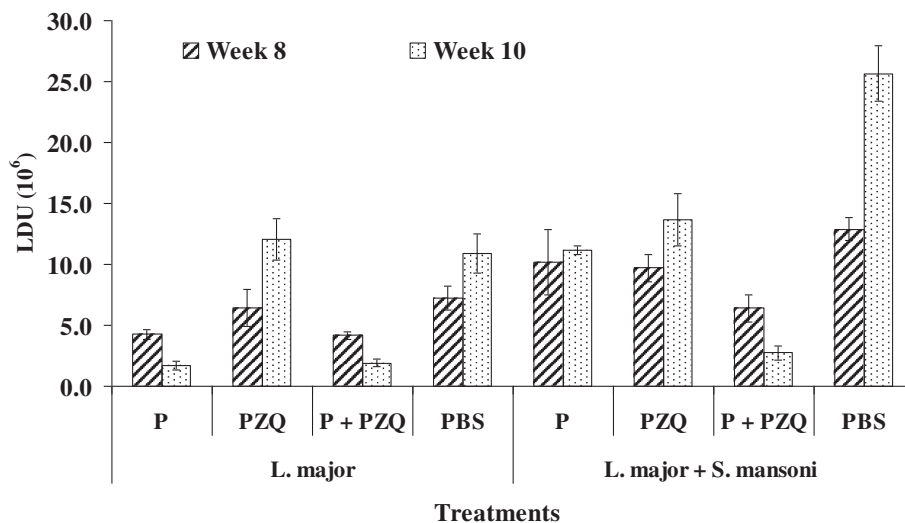
Infection	Drug administration	Spleno-somatic index		Hepato-somatic index	
		Week 8	Week 10	Week 8	Week 10
<i>L. major</i>	P	0.77 ± 0.031	0.74 ± 0.039	4.45 ± 0.14	3.92 ± 0.08
	PZQ	1.11 ± 0.173	1.45 ± 0.100	4.67 ± 0.15	5.18 ± 0.23
	P + PZQ	0.81 ± 0.016	0.74 ± 0.029	4.25 ± 0.18	3.81 ± 0.13
	PBS	1.03 ± 0.150	1.67 ± 0.084	4.57 ± 0.10	5.97 ± 0.32
<i>S. mansoni</i>	P	1.11 ± 0.088	1.49 ± 0.066	8.83 ± 1.11	10.19 ± 0.50
	PZQ	0.94 ± 0.073	0.96 ± 0.064	4.61 ± 0.09	4.14 ± 0.21
	P + PZQ	0.86 ± 0.081	0.79 ± 0.044	4.46 ± 0.07	4.04 ± 0.22
	PBS	1.09 ± 0.081	1.72 ± 0.073	5.87 ± 1.12	7.66 ± 0.27
<i>L. major</i> +	P	1.11 ± 0.165	1.37 ± 0.076	7.83 ± 1.44	8.68 ± 2.04
<i>S. mansoni</i>	PZQ	1.20 ± 0.111	1.41 ± 0.077	8.64 ± 0.12	9.47 ± 0.13
	P + PZQ	1.03 ± 0.075	1.01 ± 0.041	4.46 ± 0.11	4.19 ± 0.07
	PBS	1.17 ± 0.046	1.88 ± 0.051	5.81 ± 0.16	7.50 ± 0.43

with *L. major* + *S. mansoni*, treatment with P + PZQ reduced LDU significantly ( $p < 0.05$ ). In the rest of the treatments there were significant ( $p < 0.05$ ) increase in LDU over the two weeks time.

Trends in the worm counts between *S. mansoni* infected and those co-infected with *L. major* + *S. mansoni* were similar (Table 3). In the Pentostam treated and control groups, there was a significant ( $p < 0.05$ ) increase in the worm counts between week 8 and week 10. In *S. mansoni* infected BALB/c mice, treatment with PZQ and P + PZQ resulted in reduced worm counts. In *L. major* + *S.*

*mansoni* co-infected BALB/c mice, treatment with P + PZQ resulted in a significant ( $p < 0.05$ ) reduction in worm counts as compared to mice treated with PZQ alone.

In the *S. mansoni* and *L. major* + *S. mansoni* infected BALB/c mice, there was a significant ( $p < 0.05$ ) reduction in the % worm recovery when PZQ and P + PZQ was applied in managing the infection albeit there was no significant ( $p > 0.05$ ) difference observed when Pentostam was used (Figure 3a). Subsequently, % worm reduction increased when PZQ and P + PZQ was administered in the treatment (Figure 3b).



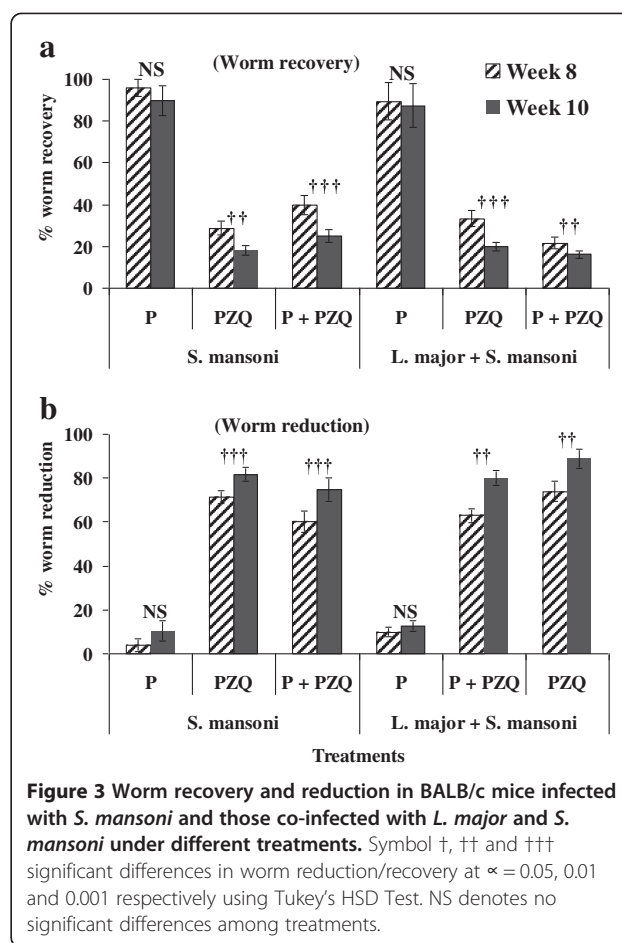
**Figure 2** The LDU of *L. major* parasite in BALB/c mice infected with *L. major* and those co-infected with *L. major* and *S. mansoni* receiving various treatments of Pentostam and PZQ between week 8 and week 10.

**Table 3 Worm counts of BALB/c infected with *S. mansoni* and those co-infected with *L. major* + *S. mansoni* under various treatments with Pentostam and PZQ between week 8 and week 10**

Infection	Drug administration	Week 8	Week 10
<i>S. mansoni</i>	P	34.80 ± 6.09	41.2 ± 2.58
	PZQ	10.4 ± 1.29	8.4 ± 1.50
	P + PZQ	14.4 ± 1.24	11.60 ± 1.24
	PBS	36.20 ± 5.17	46 ± 2.79
<i>L. major</i> + <i>S. mansoni</i>	P	30.60 ± 7.59	38.6 ± 2.68
	PZQ	11.40 ± 2.74	8.80 ± 1.44
	P + PZQ	7.40 ± 2.60	5.0 ± 1.20
	PBS	34.20 ± 3.14	44.2 ± 2.37
<b>Friedman Two-Way ANOVA</b>	<b>Main effect</b>		
	F	11.231	19.258
	P-value	0.0002	0.0000
	<b>Repeated measure</b>		
F		7.511	
P-value		0.0014	

### Discussion

The drugs recommended in the management of *Leishmania* are pentavalent antimonials such as Pentostam, while management of *Schistosoma* is done using PZQ. Yet, the interactions among parasitic agents in the hosts may alter the epidemiology, development and severity of the disease [1] with unknown consequences on the efficacy of the combined first line treatments. The aim of the present study was to evaluate the efficacy of combined first line of treatment in managing co-infection of *L. major* and *S. mansoni* in BALB/c mice. There was no evidence of antagonistic effects of PZQ on Pentostam, or a possible additive and/or synergistic effect on Pentostam during *Leishmania* mono-infection treatment, which may be associated with PZQ and Pentostam distinct targets and mode of action [36-40,57,58]. Studies in murine models indicate concurrent infection of *L. major* and *S. mansoni*, exacerbate lesion size and/or inability to resolve footpad lesions as rapidly as mice infected with *L. major* which correlates with the impaired ability to rescind parasitic burden [22]. Therefore, it is apparent that a strategy to enhance the resolution of infection in mice is through targeting the *S. mansoni*. In this study, when we applied combined chemotherapeutics of first line treatment for *S. mansoni* (PZQ) and *L. major* (Pentostam) comorbidity, there were large reductions of lesion size in the mice compared to the reduction in lesion size in BALB/c treated with Pentostam or PZQ alone. This observation reveals that through action on *S. mansoni*, the Pentostam acts much faster to heal the lesion in BALB/c mice. Previously, pre-infection with *S. mansoni* delayed the resolution of *L.*



*major* lesions while *L. major* infection had no impact on the course of *S. mansoni* infection in co-infected mice [24]. Since the granuloma response by *S. mansoni* forms a discrete niche that facilitates the intracellular survival of *Leishmania* [26], the reduction of *S. mansoni* through the first line of treatment could enhance the healing response in co-infected mice. It is also probable that healing in combined therapy treatment was achieved through immune response pathways [39,57-60]. Nonetheless, the co-infected BALB/c mice under mono-therapy with Pentostam had significantly smaller lesion nodules as compared to the PZQ treated mice that had no significant difference between the start of treatment and after treatment. Although subject to further scrutiny, it would be possible that the divergence would be explained by having the two arms of immunity operational at the same time. Treatment of *S. mansoni* infection using PZQ has been demonstrated to result in better Th2 responses thus limiting Th1 responses, which tend to abrogate *Leishmania* infection [24,25,34,39,40,58,61-63].

Co-infection may cause an increased stress response in mice manifested through reduced physiological functioning and resulting in reduced growth response [64].

Body weights exhibited growth response to treatments in the current study. The largest increase in body weight occurred in mice infected with *L. major* and treated with Pentostam, and in *S. mansoni* infected mice and treated with PZQ, suggesting efficacy of the first line treatments against mono-parasite infections as previously determined [48,50,60-63]. However, the lower percentage increase in body weight of the *L. major* infected mice after treatment with P + PZQ suggest a more pathological response of BALB/c mice to one of the drugs indicating that combined therapy may tend to be more toxic to mice infected by one parasite than single therapy administration. Although pathological effects of combined Pentostam and PZQ has rarely been shown in co-infected mice, we observed that between week five and six there were signs of toxicity [65] in BALB/c mice co-infected with *L. major* and *S. mansoni* and subjected to concurrent treatment with P + PZQ. In the co-infected mice, the use of combined therapy was found to result in a slight increase in the body weight, which may be concomitant to normal somatic growth. The reduced body weights in BALB/c mice co-infected with *L. major* and *S. mansoni* and treated with either Pentostam or PZQ therapies alone suggest that elimination of one parasite by the single drug does not preclude the pathological impacts of the other parasites resulting in physiological impairment of the mice and hence the observed reduced body weight.

Higher splenomegaly has been previously associated with high parasite burden in co-infected conspecifics [22]. Naturally therefore, the elimination of the parasite tends to reverse the condition. The reduction in the weight of spleen in BALB/c infected with *L. major* and *S. mansoni* and treated with Pentostam and PZQ respectively confirm the efficacy of the first line treatment drugs on these two parasites, which appeared to reduce splenomegaly. However, the non-significant increase in the weight of the spleen between week 8 to 10 suggested efficacious control of the parasites and the increased weight of the spleen was a response to normal somatic growth.

In diverse pathogenesis and pathology findings, infection with the parasitic helminth *S. mansoni* is predominantly associated with liver enlargement resulting in hepatomegaly, which although less severe, has been shown in the presence of *L. major* infection in murine models and hence possible aggravated hepatomegaly in concomitance of the two parasitic diseases [22,66-70]. In the present study, chemotherapy with first line treatments for *L. major* or *S. mansoni* single infection reduced the liver weights between week 8 and 10 suggesting reduced incidence of hepatomegaly which concurs with previous findings [50,71]. The increased liver weight suggests higher incidences of hepatomegaly in the co-infected mice when monotherapy was used as treatment modality. Indicating

that modulation of hepatomegaly requires the elimination of both parasites from the BALB/c mice. We confirmed in the present study that when combined therapy of P + PZQ was applied during co-infection, there were non-significant increases in the liver weights between week 8 and 10 pointing to elimination of the parasites that may cause hepatomegaly.

The study also established that in BALB/c mice co-infected with *L. major* + *S. mansoni*, there was a significant reduction in LDU and *S. mansoni* worm counts between week 8 and 10 when treatment was carried out using P + PZQ. The results concur with previous findings that demonstrated respective chemotherapy for the helminth or protozoan parasites at curative doses effectively reducing parasitic loads; even in the absence of total elimination of the parasites at curative or subcurative dosages particularly in parasitic helminth infection, the need for repeated chemotherapy to attain optimal therapy is considered to be core [41,49,50,69,72-74]. However, when combination therapy was used during the co-infection, signs of toxicity were noted at the start of the treatment, perhaps due to accumulation of the drugs in the tissues, particularly in liver and spleen and associated Pentostam reversible cardio toxicity among other side effects of PZQ and Pentostam previously reported [37-40,75]. The reduction of parasitic load in the current study in the combined therapy may reflect the mode of action of the drugs. Pentostam acts upon several targets that include influencing the bioenergetics of *Leishmania* parasite by inhibiting parasite glycolysis, fatty acid beta-oxidation and inhibition of ADP phosphorylation [36,37,57]. Praziquantel produces a well-documented effect on intracellular  $Ca^{2+}$  levels in adult schistosomes; within 5 minutes of exposure to the drug, adult schistosomes exhibit a rapid sustained contraction of the worm's musculature and vacuolization and disruption of the parasite tegument, an effect associated with the subsequent exposure of parasite antigens on the surface of the worm [58].

For a long time, treatment of parasite infections in murine models has focused on the single therapy treatments which use first line treatment that is generally acceptable. Recent cases of increased incidences of polyparasitism in vertebrates call for better ways to manage co-infections. These notwithstanding, interactions between co-infecting parasites that have pronounced consequences for disease development, severity and transmission dynamics may complicate the management of concomitance. The results of the present study demonstrate that the use of combined therapy resulted in management of *Leishmania* and *Schistosoma* in BALB/c mice.

## Conclusions

In the present study, treatment of BALB/c mice co-infected with *L. major* and *S. mansoni* was achieved



through combination therapy using first line treatments of Pentostam and Praziquantel, representing the first line treatments of *Leishmania* and *Schistosoma* respectively. Combined therapy resulted in reduced lesion size, low parasite burden and appeared to have fewer effects on the physiology of the BALB/c mice. Although most of the parasitic infections are managed as single infections, the problem of co-infection among patients is increasingly being recognized. The results obtained demonstrate that management of co-infections can be achieved by appropriate application of combined therapy of first line treatment, which will go a long way in the management of some of the most neglected tropical diseases.

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

This work was carried out in collaboration between all authors. CKW, VCSN, COA and HLM conceived and designed the study. CKW, JI, LWK, KSG and BJ performed the experiments. COA, CKW, JI and O-OE contributed reagents/materials/analysis tools and logistical support. CKW, O-OE and COA analyzed the data. All the authors participated in drafting and revising the manuscript. All authors read and approved the final manuscript.

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