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ORIGINAL ARTICLE

Effect of nanoparticles on the therapeutic efficacy of praziquantel against *Schistosoma mansoni* infection in murine models

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Abstract Praziquantel (PZQ) is the main treatment of Schistosomiasis mansoni. However, resistance to it was described. So, there is a necessity to develop novel drugs or to enhance the present drugs. This work aimed to assess the efficacy of PZQ alone and when loaded on liposomes in treatment of *S. mansoni* infection by parasitological and histopathological studies in experimental murine models. 112 male laboratories bred Swiss Albino mice were used in this work. They were divided into four groups: Group 1: control group; Group 2: infected then treated by PZQ (500 mg/kg) at 7, 30 and 45 days post infection; Group 3: infected then treated by liposome encapsulated PZQ (lip.PZQ) (500 mg/kg) at 7, 30 and 45 days post infection; Group 4: infected then treated by free liposomes at 7, 30 and 45 days post infection. The results showed that G3 caused the highest significant reduction of the total worm count, eggs/gram liver tissue and intestine (97.2%, 99.3%, 99.5%) respectively. Followed by G2 (85.1%, 97.6%, 89.8%) respectively. Regarding the histopathological studies, G3 showed the highest significant reduction in number and diameter of hepatic granuloma (97.6% and 98.1%), followed by G2 (77.2% and 75%) when compared to other groups. In conclusion, lip.PZQ is more effective than free PZQ from all aspects especially when administered 45 days PI.

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Introduction

Schistosomiasis is one of the widely distributed parasitic diseases which have serious health problems. Its prevalence is increasing in developing countries due to the poor socioeconomic status, poor personal and environmental hygiene and recurrent open water contact habits (WHO 2017). It is the 2nd most serious disease after malaria. So, Schistosoma control persists a challenge in endemic areas (Vos et al. 2012). It was estimated that 200 million people are infected, 120 million of them are symptomatic, 20 million have severe disease and 600 million are at risk of infection (Adenowo et al. 2015). Health impacts caused by schistosomiasis included anaemia, difficulties in learning and malnutrition (Gazzinelli et al. 2016).

Schistosomiasis was traditionally the most significant public health problem in Egypt. Its treatment aims to reverse the acute disease and prevent the complications (WHO 2014). Praziquantel (PZQ) is the main drug used in treatment of schistosomiasis and the first antihelmenthic drug to meet WHO requirements, being well tolerated by the affected population and effective in reduction of parasitic load (Thétiot-Laurent et al. 2013). However, PZQ has failed in the last years, this was approved by detecting eggs in post-treatment stool examinations. This failure is due to its low bioavailability (Légaré and Ouellette 2017). Due to its significance in combating against the disease, several studies have focused on improving its solubility and enhacement of its delivery systems (de Pádua Oliveira et al. 2016).

Liposomes are minute vesicles composed of multiple spheres of lipid bilayers and separated from each other. Their size ranged from 80 to 100 nm (Mourão et al. 2005). Lipid-based delivery systems, such as liposomes, are found to be important routes in the oral delivery of drugs because they can incorporate hydrophobic and hydrophilic drugs and they haven't any toxicity (Bozzuto and Molinari 2015). Several studies showed that the oral drug delivery by liposomes helps it to reach to the target sites in the organism, rapid solubility and bioavailability with reduction in its toxicity (Akbarzadeh et al. 2013). Since liposomal composition is similar to the targeted cell membrane, an enhanced lipid–lipid exchange occurs. This leads to rapid release of lipophilic drugs from the liposomal lipid layer into the targeted cell membrane (Pattni et al. 2015).

The current work aimed to assess the role of nanotechnology-based drug delivery systems in combating *S. mansoni* infection through the use of phosphatidylcholine (PC) liposomes containing PZQ to evaluate and compare the efficacy of PZQ alone and when loaded in liposomes in treatment the infection. This assessment is done by parasitological and histopathological studies in experimental murine models.

Materials and methods

Non-randomized control-study was started on November 2017 till August 2018 and was performed in the laboratories of Medical Parasitology Department, Faculty of Medicine, Zagazig University; Medical Research Institute, Alexandria University; Faculty of Science, Alexandria University; Faculty of Pharmacy, Alexandria University; and Theodor Bilharz Research Institute (TBRI), Imbaba, Giza, Egypt.

Experimental animals

One hundred and twelve Swiss albino mice of CDI strain were used, about eight weeks old, (18–20 g) at the beginning of the experiment. The animals were maintained in well-ventilated plastic cages in an air-conditioned room at 22 °C.

Ethical aspects

All procedures related to animal experimentation in the present study met the International Guiding Principles for Biomedical Research Involving Animals as issued by the International Organizations of Medical Sciences and approved by ethics committee of the Faculty of Medicine, Zagazig University.

Experimental infection

Cercariae of *S. mansoni* virulent strain were obtained from infected *B. alexandrina* snails, which were reared and maintained at Schistosoma Biological Supply Center (SBSP), TBRI. Infection of mice was done subcutaneously according to the method of Peters and Warren (1969) by a dose of 60 ± 10 cercariae/mouse. 0.2 ml of the cercaial suspension was mixed, and then was withdrawn to be injected subcutaneously into the loose skin of the back of the mouse by an insulin syringe (Liang et al. 1987).

Drug preparation and administration

1. *PZQ*: PZQ powder (active ingradient) (Distocide®, EIPICO, 10th of Ramadan, Egypt). PZQ was given by oral route in a form of a suspension in 2% (v/v) cremophore-El (Sigma-Aldrich Chemical Co, St. Louis, MO). The drug was taken orally in a dose of 500 mg/kg (1000 mg/kg total dose) (Gönnert and Andrews 1977).
2. *Preparation of liposomes*: Liposomes were synthesized in Medical Bio-Physics Department, Medical Research Institute, Alexandria University by "thin film hydration method" according the method of Cinto et al. (2009).
3. *Preparation of PZQ- loaded liposomes*: Firstly, dissolving of the drug in the phosphatidylcholine (PC)-chloroform solution, in a molar ratio PC/PZQ equal to 6:1. The usage of this ratio was due to the loading capacity of PZQ, without precipitation; in PC liposomes is 1: 5 PZQ/PC molar ratio (Mourão et al. 2005). The morphology and dimensions of the liposomal PZQ (Lip.PZQ) nanosuspension was detected via transmission electron microscopy (TEM; JEM-1400 Plus; JEOL, Japan) of the liquid containing the dispersed nanosuspension was placed on the copper grid for TEM examination. This work was performed in Electron Microscope Unit, Faculty of Science, Alexandria University. **Determination of Entrapment Efficiency of liposomes** using the equation:

$$\text{Entrapment efficiency (EE)} = (\text{amount of bound drug}/\text{total amount of drug}) \times 100$$

Grouping of animals

Mice were subdivided into nine treated groups (10 mice in each group) and a control group of twenty-two mice. Group 1 (control group) was subdivided into Group (1a) control normal group: consisting of twelve mice and group (1b) control infected: consisting of ten mice.Group 2 was subdivided into (2a); (2b) and (2c) subgroups which were

infected then treated by PZQ (500 mg/kg) at 7, 30 and 45 days post infection respectively. Group 3 was subdivided into (3a) and (3b) and (3c) subgroups which were infected then treated by Lip.PZQ (500 mg/kg) at 7, 30 and 45 days post infection respectively. Group 4 was subdivided into (4a) and (4b) and (4c) subgroups which were infected then treated by free liposomes at 7, 30 and 45 days post infection respectively.

Drug administration regimens

The control group was divided into, one control normal group (control negative) and infected control group (control positive). Groups 2a, 3a and 4a were treated by the drug on the 7th day post infection to assess its effect on schistosomula. Groups 2b, 3b and 4b were treated on the 30th day post infection to assess its effect on juvenile stages. Groups 2c, 3c and 4c were treated on the 45th day post infection to assess its effect upon mature adult stages. One concentration of both PZQ and encapsulated PZQ (lip.PZQ) was used (500 mg/kg). PZQ was given to thirty mice in the treatment group; also, the same dose of Lip.PZQ was given to another thirty mice; last thirty animals received free liposomes; lastly, 10 mice were given Phosphate buffer (20 mM, pH 7.4) while the negative control group consisting of twelve mice received nothing.

Eight weeks post infection mice were sacrificed for different evaluations using rapid decapitation. According to the method of Duvall and DeWitte (1967), Porto- mesenteric perfusion was performed.

Parasitological studies

The determination of worm burden was done by perfusion of the Porto-mesenteric system. The number of eggs per gram intestine and liver was calculated after weighting and digestion of all pieces of intestine and liver of sacrificed infected mice in 5% KOH solution (Cheever 1968).

Histopathological studies

After extraction, collection of parts of liver was done from each mouse, followed by fixation at 10% buffered formalin solution, and then prepared as paraffin wax blocks.

Counting of granuloma numbers and measurement of granuloma diameters in liver sections stained with hematoxylin and eosin (H&E)

Haematoxylin and eosin-stained liver sections of 4-μm-thickness were examined and counting the number. According to Ali and Manal (2006), and the diameter of hepatic bilharzial granulomas was measured (Romeih et al. 2008). The granulomas were photographed and counted by Leica Image Manager 50/4.0 software.

Statistical analysis

Data analysis was performed using the software SPSS (Statistical Package for the Social Sciences) version 20.

Results

Characterization of liposomes

TEM study of liposomes and PZQ-loaded liposomes

TEM micrographs showed that liposomes were formed as discrete spherical vesicles with sizes below 200 nm. Plain liposomes appeared as hollow spheres. However, PZQ-loaded particles were larger in size than their corresponding plain liposomes. This increase in size pointed to successful drug encapsulation into the core of liposomes as shown in Figs. 1 and 2.

The entrapment efficiency depends on physicochemical properties of the drug, lipid composition, charge on the lipid and amount of cholesterol used in the formulation. The absorbance intensity of unloaded PZQ in the supernatant was 0.251 at λ_{max} 262 nm. The concentration corresponding to this absorbance was 10.0 mg/ml as calculated from the standard curve. The entrapment efficiency of PZQ was expressed as a ratio between the concentration of PZQ in the liposomes and the concentration of PZQ added to the system according to the following equation:

$$EE = [(Dt - Du)/Dt] \times 100 = [(218 - 10)/218] \times 100 = 95\%$$

Dt total drug amount, *Du* uncombined (free) drug.

All mice groups were sacrificed eight weeks post infection for parasitological and histopathological evaluations, the collected data were analyzed and presented in the following tables and figures.

Parasitological parameters

Table 1 showed that treatment with Lip.PZQ had the highest reduction in the mean total worm burden when given 30 and 45 days PI compared to their corresponding control infected, PZQ and free liposomes groups while the reduction in the mean total worm count was non-significant when given 7 days PI compared to infected control group (Fig. 3).

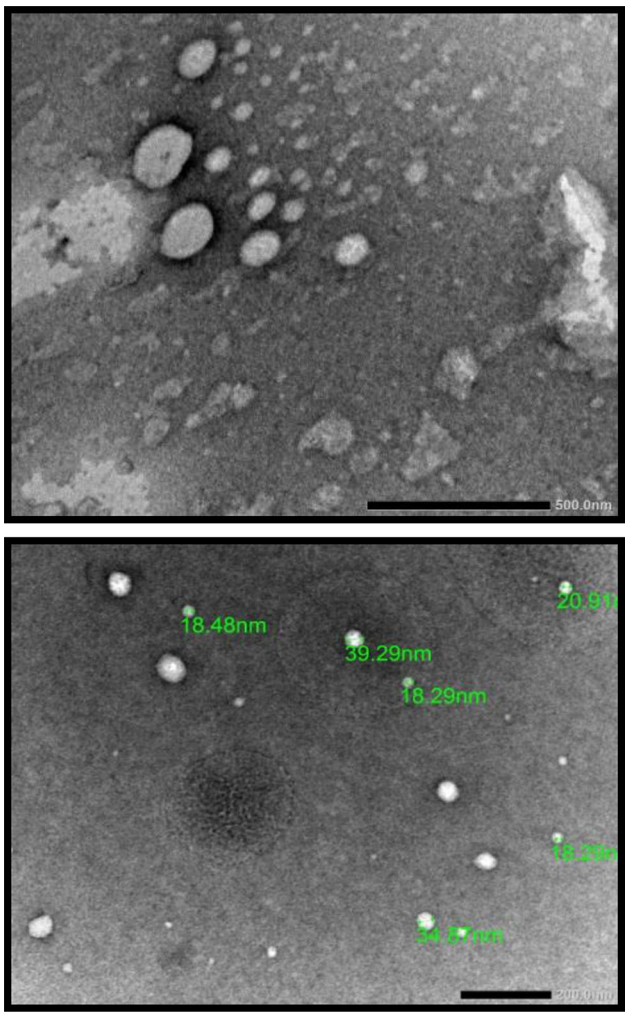

Fig. 1 TEM micrographs of plain liposomes

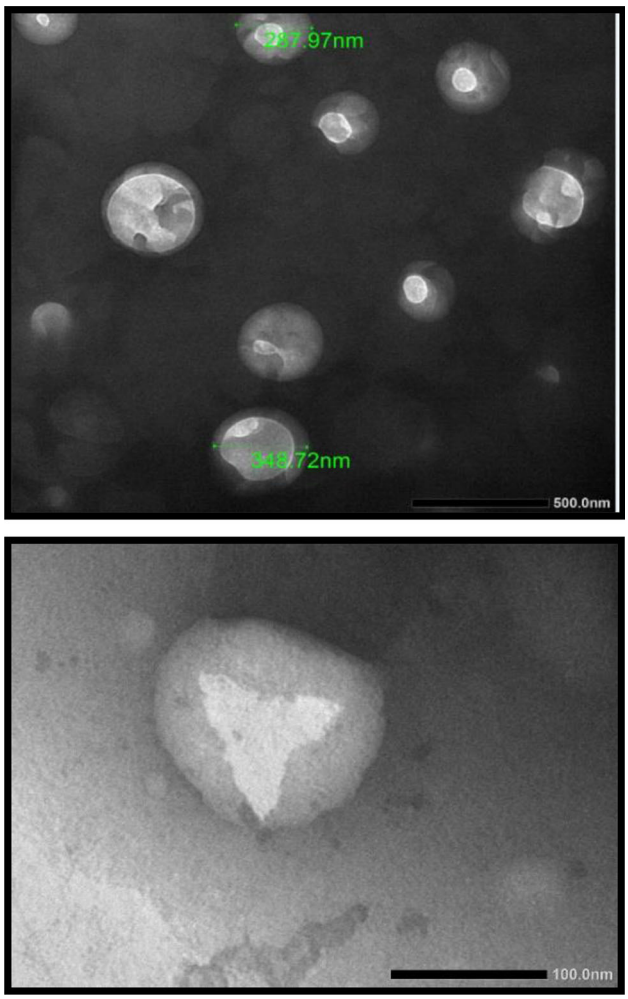

Fig. 2 TEM micrographs of PZQ- loaded liposomes entrapment efficiency (EE%)

PZQ given at 30 and 45 days PI showed significant reduction in mean total worm count while at 7 days PI, it had insignificant effect compared to infected control group.

On the contrary, there was non-significant reduction in mean total worm count in groups treated with free liposomes at 7, 30 and 45 days PI compared to infected control group.

Table 2 showed that treatment with Lip.PZQ had the highest reduction in the mean egg count/gram liver when given 30 and 45 days PI compared to their corresponding infected control, PZQ and free liposomes groups however the reduction in mean egg count/gram liver at 7 days PI was non-significant. PZQ given at 30 and 45 days PI had significant reduction in mean egg count/gm liver while at 7 days PI, it had non-significant effect compared to infected control group. Also, there was non-significant reduction in mean egg count/gram liver in groups treated with free liposomes at 7, 30 and 45 days PI compared to infected control group.

Table 3 showed that treatment with Lip.PZQ had the highest reduction in the mean egg count/gram intestine when given 30 and 45 days PI compared to their corresponding infected control, PZQ and free liposomes groups however it had insignificant effect when given at 7 days PI compared to infected control group. PZQ given at 30 and 45 days PI, had significant effect on reduction of mean egg count/gram intestine while at 7 days PI, it had insignificant effect in comparison to infected control group. Also, there was insignificant reduction in mean egg count/gram intestine in groups treated with free liposomes at 7, 30 and 45 days PI compared to infected control group.

Histopathological results

Table 4 showed that treated group with Lip.PZQ had the highest reduction in the mean number of liver granuloma when given 30 and 45 days PI compared to their corresponding infected control, PZQ and free liposomes groups,

Table 1 Effect of PZQ, Lip.PZQ and free liposomes on total worm count in *S. mansoni* infected mice treated at 7, 30 and 45 days PI

	Control group	PZQ Mean ± SD (R%)	Lip.PZQ Mean ± SD (R%)	Liposomes Mean ± SD (R%)	F	*p*
7 days	14.1 ± 1.97	13.2 ± 1.32 (6.4%)	12.3 ± 1.49 (12.8%)	13.5 ± 1.9 (4.25%)	1.964	0.137
30 days		6.6 ± 1.84 (53.2%)	3.5 ± 0.71 (75.2%)	12.3 ± 1.16 (12.8%)	106.736	< 0.001**
45 days		2.1 ± 0.74 (85.1%)	0.4 ± 0.7 (97.2%)	12.9 ± 1.97 (8.5%)	KW 32.940	< 0.001**
p		< 0.001**	< 0.001**	0.674		

**Highly significant

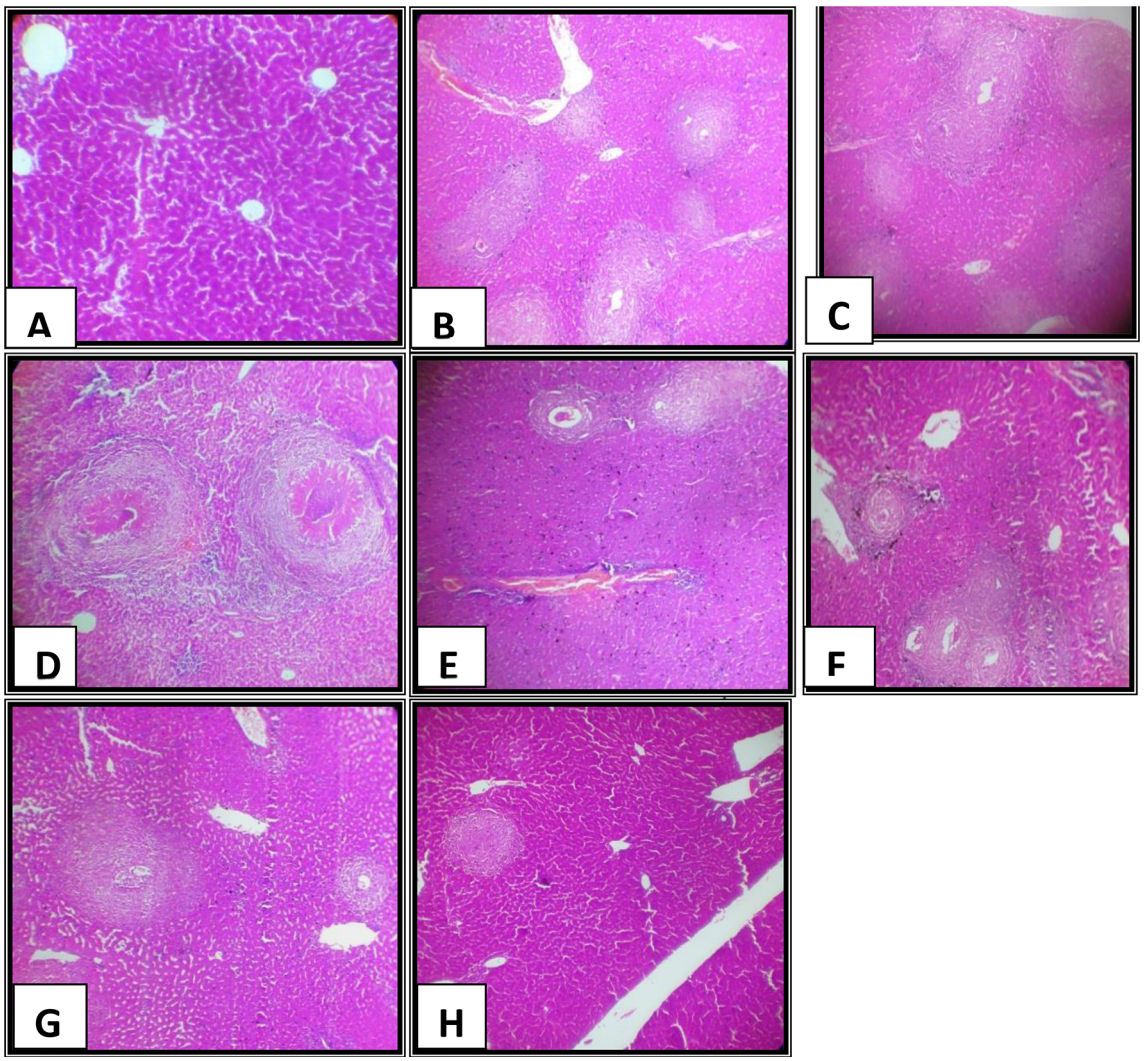

Fig. 3 Liver sections (H&E X100) **a** control healthy. **b** *S. mansoni* infected control with multiple bilharzial granulomas. **c** *S. mansoni* infected mice treated with PZQ 7 days PI showing multiple bilharzial granulomas. **d** *S. mansoni* infected mice treated with PZQ 30 days PI showing two large bilharzial granulomas with aggregates of inflammatory cells. **e** *S. mansoni* infected mice treated with PZQ 45 days PI showing two small bilharzial granulomas. **f** *S. mansoni* infected mice treated with Lip.PZQ 7 days PI showing multiple bilharzial granulomas with aggregates of inflammatory cells. **g** *S. mansoni* infected mice treated with Lip.PZQ 30 days PI showing three bilharzial granulomas with moderate aggregates of inflammatory cells. **h** *S. mansoni* infected mice treated with Lip.PZQ 45 days PI showing one bilharzial granuloma with few aggregates of inflammatory cells

Table 2 Effect of PZQ, Lip.PZQ and free liposomes on egg count/gram liver in *S. mansoni* infected mice treated at 7, 30 and 45 days PI

	Control group	PZQ Mean ± SD (R%)	Lip.PZQ Mean ± SD (R%)	Liposomes Mean ± SD (R%)	F	*p*
7 days	6995.2 ± 1309.65	6904.3 ± 1324.9 (1.3%)	6796.2 ± 1309.65 (2.8%)	6961.3 ± 1338.4 (0.5%)	0.047	0.986
30 days		4746 ± 802.49 (32.2%)	2419 ± 479.71 (65.4%)	6750.4 ± 1361.7 (3.5%)	53.53	<0.001**
45 days		170.9 ± 30.64 (97.6%)	47 ± 13.69 (99.3%)	5872.6 ± 526.1 (16%)	271.89	<0.001**
p		<0.001**	<0.001**	0.319		

**Highly significant

Table 3 Effect of PZQ, Lip.PZQ and free liposomes on egg count/gram intestine in *S. mansoni* infected mice treated at 7, 30 and 45 days PI

	Control group	PZQ Mean ± SD (R%)	Lip.PZQ Mean ± SD (R%)	Liposomes Mean ± SD (R%)	F	*p*
7 days	10,485.8 ± 2863	9793.7 ± 1279.1 (6.6%)	9720.5 ± 808.06 (7.3%)	10,461 ± 712.39 (0.2%)	2.1	0.117
30 days		5968.7 ± 1338.66 (43%)	3232.6 ± 538.45 (69.2%)	10,461 ± 632.13 (0.2%)	47.72	<0.001**
45 days		1068.8 ± 709.56 (89.8%)	56.9 ± 13.56 (99.5%)	10,461 ± 712.39 (0.2%)	144.9	<0.001**
p		<0.001**	<0.001**	0.187		

**Highly significant

Table 4 Effect of PZQ, Lip.PZQ and free liposomes on mean number of liver granuloma in *S. mansoni* infected mice treated at 7, 30 and 45 days PI

	Control group	PZQ Mean ± SD (R%)	Lip.PZQ Mean ± SD (R%)	Liposomes Mean ± SD (R%)	F	*p*
7 days	12.3 ± 2.5	11.6 ± 2.54 (5.7%)	10.7 ± 2.4 (13%)	12.2 ± 2.1 (0.8%)	1.523	0.225
30 days		4.8 ± 1.3 (61%)	3.3 ± 0.8 (73.2%)	12.2 ± 1.62 (0.8%)	79.35	<0.001**
45 days		2.8 ± 0.9 (77.2%)	0.3 ± 0.5 (97.6%)	12.2 ± 1.57 (0.8%)	158.43	<0.001**
p		<0.001**	<0.001**	0.571		

**Highly significant

while the reduction in mean number of liver granuloma was insignificant in the same group at 7 days PI compared to infected control group. PZQ given at 30 and 45 days PI had significant reduction in mean number of liver granuloma while at 7 days PI; it had insignificant effect compared to infected control group. Also, there was insignificant reduction in mean number of liver granuloma in groups treated with free liposomes at 7, 30 and 45 days PI compared to infected control group.

Table 5 showed that treatment with Lip.PZQ had the highest reduction in the mean diameter of liver granuloma when given 30 and 45 days PI compared to their corresponding infected control, PZQ and free liposomes groups. While, there was insignificant reduction in mean diameter of liver granuloma when Lip.PZQ was given at 7 days PI compared to infected control group. PZQ had significant reduction in mean diameter of liver granuloma when given at 30 and 45 days PI while it had insignificant effect when given at 7 days PI compared to infected control group. Also, there was insignificant reduction in mean diameter of liver granuloma in groups treated with free liposomes 7, 30 and 45 days PI compared to infected control group.

Discussion

PZQ is the main drug for the treatment of schistosomiasis. However, several studies are needed to understand the mechanism of action and improve its bioavailability (Dömling and Khoury 2010).

Nano structured lipid systems including liposomes, solid lipid nanoparticles and solid lipid nanocarriers have focused on improving the biopharmaceutical properties of poorly water soluble drugs (Kolenyak-Santos et al. 2014).

Table 5 Effect of PZQ, Lip.PZQ and free liposomes on mean diameter of liver granuloma (μm) in *S. mansoni* infected mice treated at 7, 30 and 45 days PI

	Control group	PZQ Mean ± SD (R%)	Lip.PZQ Mean ± SD (R%)	Liposomes Mean ± SD (R%)	F	*p*
7 days	349.2 ± 78.93	336.1 ± 67.37 (3.8%)	315.3 ± 78.93 (9.7%)	339.8 ± 67.9 (2.7%)	0.407	0.749
30 days		139 ± 79.4 (60.2%)	98.2 ± 5 (71.9%)	337.4 ± 41.7 (3.4%)	50.58	<0.001**
45 days		87.5 ± 7.1 (75%)	6.7 ± 0.7 (98.1%)	337.3 ± 41.6 (3.4%)	147.6	<0.001**
p		<0.001**	<0.001**	0.798		

**Highly significant

In vivo studies have reported the improvement of the PZQ activity by the liposomes. The behavior of liposomes in biological systems and their interaction with the drug and the site of action must be understood to make liposome-encapsulated drugs available in the future (Geary 2012).

The incorporation of PZQ in small unilamellar liposomes composed of PC can allow the drug administration in an aqueous media without decreasing the effect of the drug on *S. mansoni*, since liposomes is acting on the lipophilic profile of the drug. The incorporation of PZQ in liposomes showed similar effect on *S. mansoni* cultures (Della Pepa et al. 2017).

The aim of this work wasto evaluate the role of nanotechnology-based drug delivery systems in combating *S. mansoni* infection through the use of PC liposomes containing PZQ to assess and compare the efficacy of PZQ alone and when loaded in liposomes in treatment the infection by parasitological and histopathological studies in experimental murine models. This work was based on the evaluation of their effects on the schistosomula, jeuvenile worms, adult worms, egg deposition and formation of hepatic granulomas.

The results of the present study have proved that encapsulation of PZQ into liposomes increases its solubility in aqueous systems, improves its bioavailability and may be targeting it to specific sites in the parasite. Increased efficacy of the drugs after their encapsulation with liposomes was reported by Alving (1986).

Regarding method of preparation of liposomes, the "thin film hydration method" was used in this study. This was in accordance with Aloisio et al. (2017) who used the same method for preparation while Frezza et al. (2013) used the "sonication method".

Regarding entrapment efficiency (EE), in this study the EE was (95%). This was similar to Aditya et al. (2010) who reported (97%) E.E when artemether was loaded within lipid nanoparticles. Higher EE was recorded by Venkateswarlu and Manjunath (2004) using stearylamine coated lipid nanoparticles. On the contrast, Varona et al. (2011) reported PZQ E.E of (66%), Hrčková and Velebny (1997) also reported that the EE of PZQ in liposomes ranged from 65 to 79%.

Regarding components used in preparation of liposomes, PC and cholesterol were prefered in this work, due to their easily production. This type of liposome is documented by the U.S. Food and Drug Administration (FDA) and other regulatory agencies to be "Generally Regarded as Safe" (GRAS) (Parnham and Wetzig 1993), for this reason, it is used in food products in a wide manner (Pardun 1988). No toxicity was detected in oral or intravenous administration of PC upon using it in treatment of liver diseases or reduction of plasma cholesterol (Kuntz 1989). Frezza et al. 2013 reported that the intravenous route administration of Lip. PZQ in rats was less toxic than free PZQ.

In this study, PC and cholesterol were used in a PC/cholesterol molar ratio of 3:1. However; other studies reported using PC/cholesterol ratio of 7:6 (Ammar et al. 1994).

Also, in this work, the usage of PZQ/PC molar ratio of 1:6 during loading PZQ into liposomes was in accordance with Frezza et al. (2013) who used the same PZQ/PC molar ratio 1:6 and Mourão et al. (2005) who used a molar ratio of 1:5. On the other hand, Hrčková and Velebny (1997) used a PZQ/PC molar ratio of 1:2.

The oral route was used because it is the most convenient, safest, and the most common method of adminstration (Porter et al. 2008). de Souza et al. (2014) reported that the encapsulation of PZQ into SLN could lead to an improvement of oral administration with minimal cytotoxicity events.

Mice are considered a good representative experimental model of the host susceptibility to schistosomiasis (Andrade and Cheever 1993).For this reason, mice were used as experimental animals in this study.

In this study, the parasitological effects of PZQ, Lip.PZQ and free liposomes were evaluated by assessing the worm burden and tissue egg load (liver and intestine).

Regarding worm burden, treatment with Lip.PZQ had the highest significant percentage of reduction in the total worm count when given 30 and 45 days PI (75.2% and 97.2%) respectively followed by PZQ treated group 30 and 45 days PI (53.2% and 85.1%) respectively. On the contrary, both of Lip.PZQ and PZQ had non-significant effect when given 7 days PI (12.8% and 6.4%) respectively compared to infected control group.While free liposomes resulted in non-significant reduction in the total worm count (4.25%, 12.8% and 8.5%) at 7, 30 and 45 days PI respectively when compared to their corresponding infected control group (Table 1).

This was explained by the fact that low water soluble drugs e.g.PZQ became more soluble when were carried on liposomes and when they were administered orally, liposome increases drug activity and bioavailability (Mourão et al. 2005). Also, the affinity of *S. mansoni* to phospholipids contributed to easy absorption of encapsulated drug by the worm (Frezza et al. 2013). Moreover, de Souza et al. (2014) in their in vitro study reported that worms exposed to PZQ-SLN revealed aspects similar to those incubated with empty SLN, suggesting a synergic effect of SLN and the drug.

These results agreed with Al-Noshokatty et al. (2018) who recorded that the usage of solid lipid nanoparticles loaded with PZQ in the treatment of infected rats with *S. mansoni* at 35 days PI resulting in significant reduction in the worm burden more than usage of PZQ alone at the same time.

Our results were higher than that reported by Frezza et al. (2013) who reported that Lip.PZQ resulted in (68.8%) reduction of total worm count when taken 45 days PI in *S. mansoni* infected mice and Frezza et al. (2015) who reported (41.2%) and (48%) reduction in total worm count when Lip.PZQ was given alone and combined with hyperbaric oxygen (HBO) at 45 days PI respectively compared to control infected group. El-Refai et al. (2018) reported reduction in total worm count of (68.3%) in *S. mansoni* infected group treated with PZQ at 45 days PI. Our results were in accordance with de Moraes (2012)who reported that PZQ has no effect on 7 days old schistosomula on a study evaluated the schistosomicidal activity and tegumental alterations induced by PZQ and piplartine on schistosomula.

Xiao et al. (1985) reported that jeuvenile worms (3 to 5 week-old) are considered to be less affected by PZQ than mature adult worms as they are exposed to lower concentrations of unchanged PZQ in the systemic circulation than the mature worms located in the liver.

Regarding tissue egg load in liver and intestine, it was found that treatment with Lip.PZQ at 30 and 45 days PI resulted in the highest percentage of reduction in egg count/gram liver (65.4% and 99.3%) and egg count/gram intestine (69.2% and 99.5%) followed by treatment with PZQ at 30 and 45 days PI in tissue egg load of liver (32.2% and 97.6%) and intestine (43% and 89.8%) respectively. Treatment with Lip.PZQ at 7 days PI resulted in reduction in liver egg count of (2.8%) and intestinal egg count of (7.3%) respectively while treatment with PZQ at 7 days PI resulted in a reduction in tissue egg load in liver and intestine of (1.3%) and (6.6%) respectively which was non-significant compared to infected control group. Free liposomes resulted in non-significant reduction in percentages of liver and intestinal egg count (0.5% and 0.2%), (3.5% and 0.2%) and (16% and 0.2%) at 7, 30 and 45 days PI respectively when compared to their corresponding infected control group (Tables 2, 3).

Our results was nearly similar to Frezza et al. (2013) who reported a reduction of intestinal egg count/gm at 30 and 45 days PI of (78%) and (79%) in groups treated with Lip.PZQ respectively, while Frezza et al. (2015) recorded higher percentages of reduction in intestinal egg count in groups treated with PZQ + HBO at 45 days PI (92.2%).

Dkhil et al. (2015) reported that AuNPs caused a highly significant reduction in egg density in the liver tissues of *S. mansoni* infected mice, with the highest reduction (69.8%) being recorded for a 1 mg dose of AuNPs. El-Feky et al. (2015) also reported that PZQ loaded in clay nanoformulations caused significant reduction in total tissue egg count of *S. mansoni* infected mice.

This reduction in oviposition may be explained by greater absorption of Lip.PZQ by the worm vitelline glands and ovaries which affects fecundity of female worms. Also, liposomal drug delivery system targets PZQ to liver where parasites reside in the hepatic sinusoids. Liposomes make a gradual and controlled release of PZQ which prevents the development of the parasite and thus reducing liver egg load (Katz and Coelho 2008).

Our results were similar to that recorded by El-Refai et al. (2018) who reported a reduction of (85.3%)in intestinal tissue egg load among group treated with PZQ 45 days PI while at 14 days PI, the reduction was only(10.3%) in *S. mansoni* infected mice. Also Sobhy et al. (2018) reported that in PZQ treated group 30 and 45 days PI, the liver egg percent reduction was (28.9%) and (82.2%) respectively. Also, Beshay et al. (2018) reported that treatment with PZQ 42 days PI had higher reduction percentages of tissue egg load in liver and intestine (90.5%) and (95.6%) respectively. So, PZQ acts on vitelline cells and ovaries at the same time with its action upon the tegument and muscular structure of the worm (Shaw and Erasmus 1988).

Histopathological changes in the liver of all the study groups were assessed to evaluate the therapeutic effect of PZQ, Lip.PZQ and free liposomes. Histopathological examination of the liver in the group treated with Lip.PZQ

45 days PI showed the highest percentages of reduction in granuloma number and diameter (97.6% and 98.1%) respectively with almost normal hepatic architecture and extremely few histopathological changes followed by PZQ 45 days PI in which the percentages of reduction in granuloma number and diameter were(77.2% and 75%) respectively. While Lip.PZQ treated group at 30 days PI showed percentages of reduction in hepatic granuloma number and diameter (73.2% and 71.9%) respectively which was higher than the percentages of reduction in hepatic granuloma number and diameter in PZQ treated group 30 days PI (61% and 60.2%) respectively. Treatment with Lip.PZQ and PZQ at 7 days PI showed non-significant reduction in liver granuloma number and diameter (13% and 9.7%) and (5.7% and 3.8%) respectively. Non-significant reduction were also found in the percentages of hepatic granuloma number (0.8%, 0.8% and 0.8%) and diameter (2.7%, 3.4% and 3.4%) in free liposomes treated groups at 7, 30 and 45 days PI (Tables 4, 5).

Liver granulomatous reaction around Schistosoma eggs is a main feature of *S. mansoni* infection (Andrade and Cheever 1993).Treatment with successful schistosomicidal drugs eradicates the worms and stops their egg deposition. At the same time, these drugs may modulate the host immune reaction to eggs (Warren et al. 1967).

Our results were higher than those obtained by Sobhy et al. (2018) who approved that there was significant reduction in mean number and diameter of liver granulom as seen in PZQ treated mice at 42 days PI (61.7% and 44%) compared to infected control group; Beshay et al. (2018) who recorded that the percentage of reduction in hepatic granuloma diameter in PZQ treated group at 42 days PI was (59.7%) and El-Refai et al. (2018) who found a percentage of reduction in hepatic granuloma number and diameter among group treated with PZQ at 45 days PI of (52.3% and 54.1%).

Frezza et al. (2013) reported that the highest percentage of reduction in the hepatic granuloma number was observed in Lip.PZQ treated group at 30 days PI with a reduction of (98.4%) while at 45 days PI the reduction was (83.1%). It was concluded that Lip.PZQ is more effective than free PZQ regarding the schistosomicidal potential, reduction of worm load and hepatic granuloma formation. This may be attributed to increasing of bioavailability of liposomal encapsulated drugs in the host. And better absorption by the tegument of *S. mansoni*.

Authors' contribution AEMLEG and FAM shared in the study design and research topics. SAA-R shared in the study design, in performing the laboratory work, wrote and reviewing the manuscript. TIAS shared in performing the laboratory work of this study. GMF, SMM, and NAM shared in the laboratory work, collecting references wrote and reviewed the manuscript and MAE-S shared in the laboratory work and interperation of the results.

Compliance with ethical standards

Conflict of interest The authors declared that there is no conflict of interests.

References

Adenowo A, Oyinloye B, Ogunyinka B, Kappo A (2015) Impact of human schistosomiasis in sub-Saharan Africa. Braz J Infect Dis 19(2):196–205

Aditya NP, Patankar S, Madhusudhan B, Murthy SR, Souto EB (2010) Arthemeter-loaded lipid nanoparticles produced by modified thin-film hydration: pharmacokinetics, toxicological and in vivo anti-malarial activity'. Eur J Pharm Sci 40(5):448–455

Akbarzadeh A, Rezaei-Sadabady R, Davaran S, Joo SW, Zarghami N, Hanifehpour Y, Samiei M, Kouhi M, Nejati-Koshki K (2013) Liposome: classification, preparation, and applications. Nanoscale Res Lett 8:102

AL-Noshokaty TM, Aly I, Abo-elmatty DM, Mesbah NM, Shehata AS, Etewa S (2018) Evaluation of solid lipid nanoparticles loaded with praziquantel for treatment of schistosomiasis mansoni infected rats. Global Adv Res J Med Sci 7(9):2315–5159

Ali SA, Manal AH (2006) Effect of Ailanthus altissima and Zizyphus spina Christi on bilharzial infestation in mice: histological and histopathological studies. J Appl Sci 6:1437–1446

Aloisio C, Antimisiaris SG, Longhi MR (2017) Liposomes containing cyclodextrins or meglumine to solubilize and improve the bioavailability of poorly soluble drugs. J Mol Liq 229:106–113

Alving CR (1986) Liposomes as drug carriers in leishmaniasis and malaria. Parasitol Today 2:101–107

Ammar HO, El-Ridy MS, Ghorab M, Ghorab MM (1994) Evaluation of the antischistosomal effect of praziquantel in a liposomal delivery system in mice. Int J Pharm 103:237–241

Andrade ZA, Cheever AW (1993) Characterization of the murine model of schistosomal hepatic periportal fibrosis ('pipestem'fibrosis). Int J Exp Pathol 74:195

Beshay EVN, Rady AA, Afifi AF, Mohamed AH (2018) Schistosomicidal, antifibrotic and antioxidant effects of Cucurbita pepo L. seed oil and praziquantel combined treatment for Schistosoma mansoni infection in a mouse model. J Helminthol 15:1–9

Bozzuto G, Molinari A (2015) Liposomes as nanomedical devices. Int J Nanomed 10:975

Cheever AW (1968) Conditions affecting the accuracy of potassium hydroxide digestion techniques for counting *Schistosoma mansoni* eggs in tissues. Bull World Health Organ 39:328

Cinto PO, Ribeiro Souza AL, Lima AC, Chaud MV, Gremião MPD (2009) LC evaluation of intestinal transport of praziquantel. Chromatographia 69:213

de Moraes J (2012) 'Antischistosomal natural compounds: present challenges for new drug screens', *Current topics in tropical medicine*, p. 335. ISBN: 978-953-51-0274-8, InTech Available from: http://www.Intechopen.com/books/current-topics-in-tropical-medicine/antischistosomal-naturalcompounds-present-challenges-for-new-drug-screens

de Pádua Oliveira DC, de Barros ALB, Belardi RM, de Goes AM, de Oliveira Souza BK, Soares DCF (2016) Mesoporous silica nanoparticles as a potential vaccine adjuvant against *Schistosoma mansoni*. J Drug Deliv Sci Technol 35:234–240

de Souza ALR, Andreani T, De Oliveira RN, Kiill CP, dos Santos FK, Allegretti SM, Souto EB, Chaud MV, Silva AM, Gremião MPD (2014) In vitro evaluation of permeation, toxicity and effect of praziquantel-loaded solid lipid nanoparticles against *Schistosoma mansoni* as a strategy to improve efficacy of the schistosomiasis treatment. Int J Pharm 463:31–37

Della Pepa ME, Martora F, FinamoreE Vitiello M, GaldieroM Franci G (2017) Role of nanoparticles in treatment of human parasites. Nanotechnology applied to pharmaceutical technology. Springer, Cham, pp 307–333

Dkhil MA, Bauomy AA, Diab MSM, Al-Quraishy S (2015) Antioxidant and hepatoprotective role of gold nanoparticles against murine hepatic schistosomiasis. Int J Nanomed 10:7467

Dömling A, Khoury K (2010) Praziquantel and schistosomiasis. ChemMedChem 5:1420–1434

Duvall RH, DeWitt WB (1967) An improved perfusion technique for recovering adult schistosomes from laboratory animals. Am J Trop Med Hyg 16:483–486

El-Feky GS, Mohamed WS, Nasr HE, El-Lakkany NM, el-Din SHS, Botros SS (2015) Praziquantel in clay nanoformualtion showed more bioavailability and higher efficacy in murine Schistosomiasis mansoni. Antimicrob Agents Chemother 59(6):3501–3508

El-Refai SA, Atia AF, Mahmoud SF (2018)'Effects of Callistemon citrinus aqueous extract on prepatent and patent infections with *Schistosoma mansoni* in experimentally infected mice', *Journal of helminthology*, 1-10.

Frezza TF, Gremião MPD, Zanotti-Magalhães EM, Magalhães LA, de Souza ALR, Allegretti SM (2013) Liposomal-praziquantel: efficacy against *Schistosoma mansoni* in a preclinical assay. Acta Trop 128:70–75

Frezza TF, de Souza ALR, Prado CCR, de Oliveira CNF, Gremião MPD, Giorgio S, Dolder MAH, Joazeiro PP, Allegretti SM (2015) Effectiveness of hyperbaric oxygen for experimental treatment of schistosomiasis mansoni using praziquantel-free and encapsulated into liposomes: assay in adult worms and oviposition. Acta Trop 150:182–189

Gazzinelli MF, Lobato L, Andrade G, Matoso LF, Diemert DJ, Gazzinelli A (2016) Improving the understanding of schistosomiasis among adolescents in endemic areas in Brazil: a comparison of educational methods. Patient Educ Couns 99:1657–1662

Geary TG (2012) Are new anthelmintics needed to eliminate human helminthiases? Curr Opin Infect Dis 25:709–717

Gönnert R, Andrews P (1977) Praziquantel, a new broad-spectrum antischistosomal agent. Zeitschrift für Parasitenkunde 52:129–150

Hrčková G, Velebny S (1997) Effect of praziquantel and liposome-incorporated praziquantel on peritoneal macrophage activation in mice infected with *Mesocestoides corti tetrathyridia* (Cestoda). Parasitology 114:475–482

Katz N, Coelho PMZ (2008) Clinical therapy of schistosomiasis mansoni: the Brazilian contribution. Acta Trop 108:72–78

Kolenyak-Santos F, Garnero C, de Oliveira RN, De Souza AL, Chorilli C, Allegretti SM, Longhi MR, Chaud MV, Gremião MP (2014) Nanostructured lipid carriers as a strategy to improve the in vitro schistosomiasis activity of praziquantel. J Nanosci Nanotechnol 14:1–12

Kuntz E (1989) Pilot-study with polyenylphosphatidylcholine in severe liver insufficiency. Medizinische Welt 40:1327–1329

Légaré D, Ouellette M (2017) Drug resistance assays for parasitic diseases. Antimicrobial drug resistance. Springer, Cham, pp 1409–1463

Liang YS, Bruce JI, Boyd DA (1987) Laboratory cultivation of schistosome vector snails and maintenance of schistosome life cycles. In: Proceeding of the 1st Sino-American symposium vol 1, pp 34–48

Mourão SC, Costa PI, Salgado HRN, Gremião MPD (2005) Improvement of antischistosomal activity of praziquantel by incorporation into phosphatidylcholine-containing liposomes. Int J Pharm 295:157–162

Pardun H (1988) 'Die Pflanzenlecithine. Verlag fiir die chemiscbe Industrie H. Ziolkowsky', Augsburg, pp 326–413

Parnham M, Wetzig H (1993) Toxicity screening of liposomes. Chem Phys Lipid 64(1–3):263–274

Pattni BS, Chupin VV, Torchilin VP (2015) New developments in liposomal drug delivery. Chem Rev 115:10938–10966

Peters PA, Warren KS (1969) A rapid method of infecting mice and other laboratory animals with *Schistosoma mansoni*: subcutaneous injection. J Parasitol 55(3):558–561

Porter CJ, Pouton CW, Cuine JF, Charman WN (2008) Enhancing intestinal drug solubilisation using lipid-based delivery systems. Adv Drug Deliv Rev 60(6):673–691

Romeih MH, Hassan HM, Shousha TSA, Saber MA (2008) Immunization against Egyptian *Schistosoma mansoni* infection by multivalent DNA vaccine. Acta Biochim Biophys Sin 40(4):327–338

Shaw MK, Erasmus DA (1988) *Schistosoma mansoni*: praziquantel-induced changes to the female reproductive system. Exp Parasitol 65:31–42

Sobhy MMK, Mahmoud SS, El-Sayed SH, RizkE MA, Raafat A, Negm MSI (2018) Impact of treatment with a protein tyrosine kinase inhibitor (Genistein) on acute and chronic experimental *Schistosoma mansoni* infection. Exp Parasitol 185:115–123

Thétiot-Laurent SA, Boissier J, Robert A, Meunier B (2013) Schistosomiasis chemotherapy. Angewandte Chem Int 52:7936–7956

Varona S, Martín A, Cocero MJ (2011) Liposomal incorporation of lavand in essential oil by a thin-film hydration method and by particles from gas-saturated solutions. Ind Eng Chem Res 50:2088–2097

Venkateswarlu V, Manjunath K (2004) Preparation, characterization and in vitro release kinetics of clozapine solid lipid nanoparticles. J Control Release 95(3):627–638

Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, Shibuya K, Salomon JA et al (2012) Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2163–2196

Warren KS, Domingo EO, Cowan RB (1967) Granuloma formation around schistosome eggs as a manifestation of delayed hypersensitivity. Am J Pathol 51:735

WHO (2017) Schistosomiasis retrieved on June 20, 2017. http://www.who.int/mediacenter/factsheets/fs115/en/

WHO Media centre (2014) Schistosomiasis. Factsheet N°115

Xiao SH, Catto BA, Webster LT (1985) Effects of praziquantel on different developmental stages of Schistosoma mansoni in vitro and in vivo'. J Infect Dis 151(6):1130–1137