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TREATMENT AND PROPHYLAXIS - ORIGINAL PAPER

Enhancement of the therapeutic efficacy of praziquantel in murine *Schistosomiasis mansoni* using silica nanocarrier

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Abstract

The main objective of this work is preparation of mesoporous silica nanoparticles loaded with praziquantel (PZQ-Si) in order to enhance the therapeutic efficacy of praziquantel (PZQ). Mice were experimentally infected with *Schistosoma mansoni* and treated 6 weeks post-infection with PZQ in different doses via either oral or intraperitoneal (IP) routes. PZQ in the same doses orally administered to *S. mansoni*-infected mice was used as a drug control, and infected and non-infected non-treated mice served as positive and negative controls, respectively. PZQ-Si exhibited good physicochemical attributes in terms of small uniform size (105 nm), spherical shape, and PZQ entrapment efficiency (83%). A maximum antischistosomal effect was achieved using orally administered PZQ-Si as reflected by total worm burden, tissue egg count, oogram pattern, and hepatic granuloma count and diameter. The biomarkers related to liver oxidative stress status and immunomodulatory effect (serum TNF-α and IL-10) were significantly improved. Data obtained implied that IP route was less efficacious for the delivery of PZQ-Si. Encapsulation of PZQ permits the reduction of the used therapeutic dose of PZQ. Hepatic DNA fragmentation, measured by comet assay, was significantly improved in infected mice treated with maximum dose of PZQ-Si as compared to positive or PZQ control groups. The results indicate that mesoporous silica NP is a promising safe nanocarrier for PZQ potentiating its antischistosomal, antioxidant, immunomodulatory, and anti-inflammatory action in animal model infected with *S. mansoni*. From a practical standpoint, PZQ-Si using a lower dose of PZQ could be suggested for effective PZQ antischistosomal mass chemotherapy.

Keywords Silica nanoparticles · *Schistosoma mansoni* · Histopathology · Oxidative stress

Introduction

Schistosomiasis is a tropical disease that affects more than 250 million people worldwide (Vale et al. 2017). *Schistosoma mansoni* is the most common one among the different schistosome species in Egypt (Bartneck et al. 2014).

The treatment and control of schistosomiasis rely on a single drug, praziquantel (PZQ) (Eissa et al. 2015). Unfortunately, it has been reported that PZQ has failed to give

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complete cure in some treated populations. After oral administration, PZQ has limited solubility (hydrophobic nature) and therefore undergoes slow absorption from the gut lumen (BCS class II) and shows low bioavailability as explained by Abla et al. (2017) that the rate and amount of PZQ absorbed are the main predictive factors of PZQ efficacy. After its absorption from the gastrointestinal tract, PZQ undergoes rapid extensive metabolism in the liver into inactive metabolites (first-pass hepatic metabolism), resulting in short half-life of PZQ in the circulation (Vale et al. 2017; Gouveia et al. 2018). PZQ becomes a less potent compound, with poor effect against juveniles of *S. mansoni* that are present in the systemic circulation and less exposed to the drug (Fakahany et al. 2014). Consequently, parasites that escaped the first treatment and matured after it are also responsible for recorded cases of PZQ failure, and therapeutic effect on them may be achieved by a second treatment with PZQ with more drug side effects (EL-Feky et al. 2015). Besides, there is an increasing concern about parasite resistance development against PZQ (Greenberg 2013). Therefore, there is a pressing need for the development of new modalities that can overcome limitations of PZQ-based chemotherapy (Vale et al. 2017).

Nanoparticles (NPs) can be defined as particles ranging in size from 1 to 100 nanometer (nm). The field of nanoparticle-based delivery system shows a rapid growth with development of new nanomaterials, and each one has its own advantages and localization. The current literature covers a wide range of applications about the purpose of the delivered substance being preventive, diagnostic, or corrective, the nature of the nanocarrier, and whether the data are generated through *in vitro* experimental setups or also replicated in experimental animals.

While Assis et al. (2018) utilized gold nanorods bound with a recombinant *S. mansoni* tegument protein Sm29 as a vaccination strategy to immunize mice against schistosomiasis, Dkhil et al. (2017) used gold nanoparticles themselves as curative means to modulate oxidative stress in mice spleen tissue induced by *S. mansoni* infection. Designing drug nanocarriers becomes the focus of increasing research as they have the potential power for enhancing the drug stability and elongating the duration of their therapeutic effect. After oral administration, NPs have offered a novel promising solution for the delivery of poorly water-soluble drugs as PZQ, at a therapeutically optimal rate and dose (da Fonseca et al. 2013). Nanoparticulate delivery systems allow administration of drugs through enteral or parenteral routes. Solid lipid nanoparticles (SLNs) loaded with PZQ were shown to improve the pharmaceutical and safety profiles of PZQ in vitro on *S. mansoni* culture (de Souza et al. 2014; Kolenyak-Santos et al. 2015). Other nanoparticles were also used in vitro; for example, liposome nanoparticles to deliver epiisopiloturine with promising results (Guimaraes et al. 2014) and poly(lactic-co-glycolic acid) (PLGA) nanoparticles that could be loaded with lignan (−)-6,6'-dinitrohinokinin (DNHK) for treatment of schistosomiasis.

Such in vitro results were successfully translated in *S. mansoni*-infected mice treated orally with PZQ loaded on SLNs or lipid nanocapsules (LNCs) (Radwan et al. 2019; Amara et al. 2018, respectively) that showed improved bioavailability and efficacy along with less toxicity. Although PZQ is the classical antischistosomal drug candidate, other drugs were also delivered using LNCs. Worth noting is miltefosine (MFS) where El-Moslemany et al. (2016) were able to enhance the histopathological outcome in *S. mansoni*-infected mice using oral doses of MFS-LNCs. Using different nanoparticles as drug carriers will develop new formulations of existing drugs that will be beneficial to the patients (Wang et al. 2017).

Mesoporous silica nanoparticles (MSNPs) have revolutionized nanobiotechnology by their ability to encapsulate large amounts of drug molecules. They were successfully applied for delivery of hydrophobic drugs, dramatically enhancing their dissolution rate and bioavailability after oral administration compared with crude drug material (Zhang et al. 2012). Mamaeva et al. (2013) noted that published reports demonstrate that MSNPs have low immune cell toxicity and observe accumulation in the spleen and liver, although particle size and charge influence the extent of accumulation and the distribution of particles between different organs. MSNPs showed a favorable toxicity profile and did not affect the viability of primary immune cells from the spleen in relevant concentrations (Heidegger et al. 2016). To our knowledge, this is the first study regarding the use of MSNPs as a nanocarrier for PZQ for treatment of schistosomiasis.

Improving the performance of already existing drugs is considered a strategy that sounds more beneficial from the economic point of view (Tomiotto-Pellissier et al. 2017). Taking into account the potential of MSNPs to improve the bioavailability of PZQ and short half-life in the circulation (Vale et al. 2017; Amara et al. 2018), the current study was planned to design MSNPs loaded with PZQ and to assess their antischistosomal *mansoni* experimental activity in comparison to positive and PZQ control groups. The feasibility of PZQ loaded MSNPs to achieve similar or comparable therapeutic efficacy using smaller doses of PZQ or parenteral route of administration was also investigated. Parasitological parameters, histopathological criteria, oxidative stress status, and immunomodulatory activities reflecting disease severity were investigated after treatment. Evaluation of toxicity was done in order to confidently regulate the safe use of PZQ-loaded MSNPs.

Materials and methods

Preparation of blank MSNPs

A total of 0.5 g of cetyl tri-methyl ammonium bromide was dissolved in 70 ml deionized water, followed by the addition of 30 ml of 2-ethoxy-ethanol. After complete dissolution, 0.5 ml of ammonium hydroxide (28%) was added, and the mixture was vigorously stirred. 2.5 ml of tetra-ethyl orth-silicate was then added to the mixture and vigorously stirred for 24 h. A white precipitate was collected using centrifugation at 5000 rpm for 30 min and washed with deionized water and absolute ethanol (Tan et al. 2011).

Loading of PZQ into MSNPs

PZQ solution was prepared by mixing 20 mg PZQ powder (Sigma-Aldrich) with 2 ml absolute ethanol. A total of 80 mg of the prepared blank mesoporous SiNPs were added to 8 ml of water and sonicated to be completely dissolved. The two solutions were mixed together (with ratio of PZQ:MSNPs; 1:4). The mixture was then shaken (100 rpm) at 37 °C for 24 h. PZQ-Si nanoparticles (PZQ-Si) were then precipitated using centrifugation at 5000 rpm for 30 min and washed to remove any free PZQ. Three different PZQ doses were loaded in MSNPs (Table 1) according to previously designed formula (Tan et al. 2011). The administrated dose of PZQ-Si is the equivalent for the free PZQ (Fig. 1).

Physico-chemical characterization of MSNPs before and after loading with PZQ

The morphology was characterized using transmission electron microscopy (TEM) operating at 200 kV, and the mean particle size and zeta potential were measured by zeta potential/particle sizer (NICOMPTM 380 ZLS, Santa Barbara, CA, USA) at the scattering angle of 90° at room temperature (Elbialy et al. 2014).

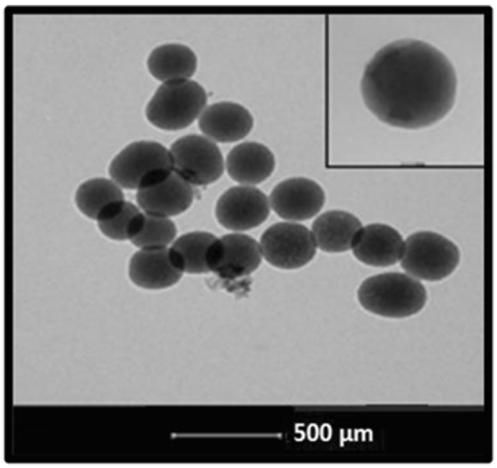

Fig. 1 Blank MSNPs under transmission electron microscopy

Entrapment efficiency (EE%) was calculated by determining the ratio of weight of PZQ entrapped into MSNPs to the total PZQ added. Serial dilutions of PZQ were prepared from the initial concentration using the equation: ($C\ V = C'\ V'$).

Where, C is the initial concentration, V is the initial volume, C' is the concentration of the prepared serial dilution, V' is the volume of the prepared serial dilution.

Absorbance of each of the prepared dilutions was measured at 260 nm, and a calibration curve was drawn. Using the calibration curve (Fig. 2), C-free (free drug concentration) was determined and EE was calculated according to the following equation:

$$EE = \frac{Initial\ concentration - Concentration\ of\ the\ free\ drug}{Initial\ concentration} \times 100$$

$$EE = \frac{C_i - C_{free}}{C_i} \times 100$$

Table 1 Study design. Mice were divided into (A) control and (B) study groups

	Animal groups (10 mice each)	Treatment
(A) Control groups	Infected non-treated control (I-C)	No
	Infected-treated with PZQ orally (I-PZQ)	250 mg/kg PZQ (I-PZQ_{250})
		500 mg/kg PZQ (I-PZQ_{500})
		1000 mg/kg PZQ (I-PZQ_{1000})
	Non-infected-non-treated (N-N)	NO
	Non-infected treated with blank MSNPs (N-MSNPs)	Unloaded MSNPs
(B) Study groups	Infected treated with PZQ-Si orally	250 mg/kg PZQ-Si (I-Oral PZQ_{250}-Si)
		500 mg/kg PZQ-Si (I-Oral PZQ_{500}-Si)
		1000 mg/kg PZQ-Si (I-Oral PZQ_{1000}-Si)
	Infected treated with PZQ-Si intraperitoneally	250 mg/kg PZQ-Si (I-IP PZQ_{250}-Si)

I-C infected control, *I* infected, *PZQ* praziquantel, *Si* encapsulated in MSNPs

Animals, parasites, and infection

Experiments were carried out on 100 male Swiss albino mice, 4–5 weeks old and weighing 20–25 g (Table 1). Cercariae of *S. mansoni* were obtained from infected *Biomphalaria alexandrina* snails. Mice and cercariae were purchased from Theodor Bilharz Research Institute (TBRI), Giza, Egypt. Each mouse was infected with 80 ± 10 *S. mansoni* cercariae by subcutaneous injection (Liang et al. 1987).

Experimental treatment

PZQ powder was freshly suspended in 2% cremophor-El before administration. Dose of the administered conventional PZQ and PZQ-Si was equally divided and administered on two consecutive days either orally or intraperitoneally. Infected groups were treated with PZQ or PZQ-Si, received treatment 6 weeks post-infection, and sacrificed 2 weeks post-treatment. Infected non-treated control group was sacrificed 8 weeks post-infection. Non-infected group treated with blank SiNPs was sacrificed 2 weeks post-treatment, and, at the same time, non-infected non-treated group was sacrificed (Fig. 3).

The anti-schistosomal effects of given treatment were assessed by the following:

A. **Parasitological parameters:** Perfusion of hepatic and porto-mesenteric vessels for estimation of total worm burden and percent worm reduction (Smithers and Terry 1965), calculation of tissue egg load in small intestine and liver (Cheever 1968), and oogram pattern (Pellegrino and Faria 1965) were performed.

B. **Histopathological parameters:** Parts of the liver of all studied groups were fixed in 10% neutral buffered formalin for histopathological study by H&E (Druray and Wallington 1980). Pathological changes in the hepatic parenchyma and the mean number and diameter of granulomata were determined (Jacobs et al. 1997).

C. **Oxidative stress markers:** Liver tissue homogenate was prepared (10% w/v) in ice-cold 0.1 M Tris–HCl buffer (pH 7.4) (Fahmy et al. 2014). Centrifugation of the homogenate was done at 3000 rpm for 15 min at 4 °C, and the resultant supernatant was used for the analysis of the following:

   i. The level of reduced glutathione (GSH) using Ellman's reagent (Ellman 1959) with some modification by Beutler et al. (1963);

   ii. The level of malondialdehyde (MDA) by colorimetric test (Ohkawa et al. 1979);

   iii. The level of nitric oxide (NO) using NO assay kit (Biodiagnostic, cat. no.: NO 25 33), according to the manufacturer's instructions (Montgomery and Dymock 1961).

D. **Immunomodulatory activity:** In vitro quantitative measurement of IL-10 and TNF-α in serum was done using sandwich enzyme-linked immunosorbent assay kit for using Cloud-Clone Corp.™ and CUSABIO™, respectively (Osmekhina et al. 2010).

E. **Genotoxicity assessment:** Single-cell gel electrophoresis (SCGE)/Comet assay was performed in liver tissue of treated mice 2 weeks post-treatment, compared with positive and negative control groups (Singh et al. 1988).

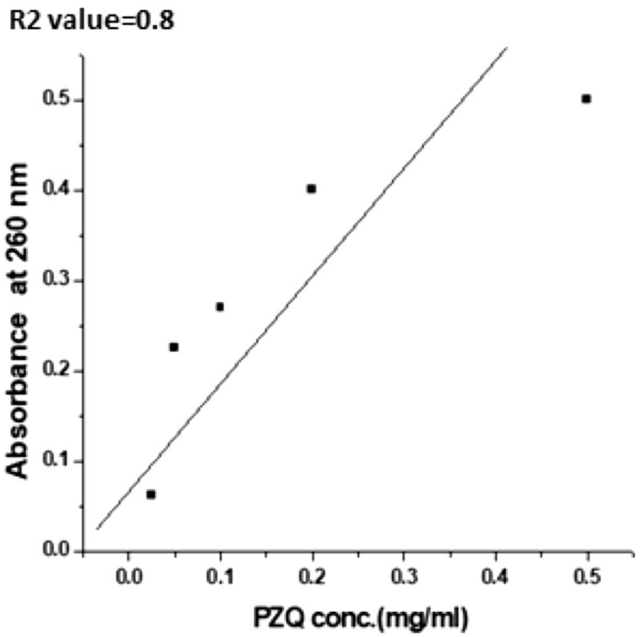

Fig. 2 Calibration curve for PZQ absorbance at 260 nm versus PZQ concentration

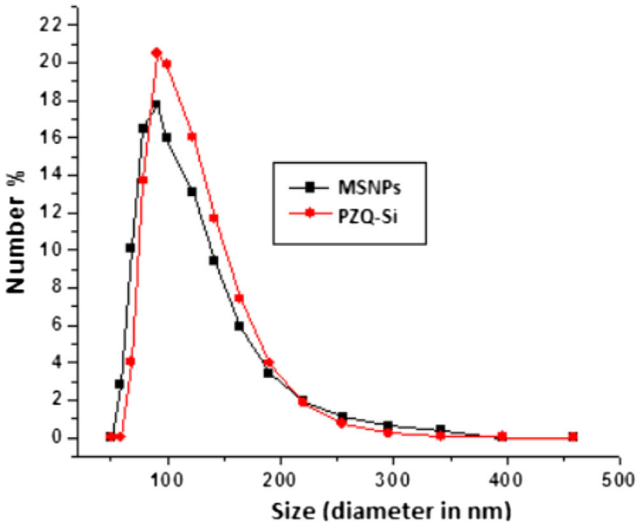

Fig. 3 Particle size distribution of PZQ-Si

Statistical analysis Recorded data were analyzed using the statistical package for social sciences, version 20.0 (SPSS Inc., Chicago, IL, USA). Quantitative data were expressed as mean ± standard error (SE). Qualitative data were expressed as frequency and percentage. A one-way analysis of variance (ANOVA) was done to compare between more than two means. Post hoc test: least significant difference (LSD) was used for multiple comparisons between different variables. The confidence interval was set to 95%, and the margin of error accepted was set to 5%. The p value ≤ 0.05 was considered significant, p value < 0.001 was considered as highly significant, and p value > 0.05 was considered insignificant (Figs. 4, 5, 6, 7, 8).

Results

Prepared blank MSNPs, under transmission electron microscopy, had nearly uniform rounded shapes with a mean diameter of 90 nm (Fig. 1). Dynamic light scattering (DLS) showed that prepared blank MSNPs have an average particle size of 100 ± 5 nm. After loading of PZQ, the size increased to 105 ± 6 nm (Fig. 3), and the surface electric charge changed from 31.9 ± 2.5 to 30 ± 2.3 mV. PZQ was encapsulated efficiently in MSNPs (83 ± 0.27%) (Table 2).

In the current study, positive control group showed active *S. mansoni* infection. Compared to positive control, PZQ either conventional or encapsulated in MSNPs in its three different doses showed effective antischistosomal effect; significantly ($p \leq 0.05$) fewer worms were recovered from the hepatic portal system, and the number of eggs retained in tissues; intestine and liver were significantly ($p \leq 0.05$) lower. A significant ($p \leq 0.05$) decrease in the percentage of immature eggs coupled with a significant ($p \leq 0.05$) increase in the percentage of dead ova was achieved. Significant ($p \leq 0.05$) reduction in the granuloma diameter and number with reduced cellularity were observed. However, PZQ-Si in its three different doses showed a greatly enhanced antischistosomal efficacy compared to that of conventional PZQ at corresponding doses. This finding was evident by remarkable and significant ($p \leq 0.05$) reduction in total tissue egg load (intestinal and hepatic) and number of immature and mature eggs associated with a significant increase ($p \leq 0.05$) in dead eggs in groups treated with 250, 500, and 1000 mg/kg PZQ loaded in MSNPs compared to groups treated with 250, 500, and 1000 mg/kg conventional PZQ, respectively (Tables 3,4). Total worm burden was significantly reduced ($p \leq 0.05$) in mice treated with PZQ-Si 250 mg/kg compared to groups treated with oral conventional PZQ 250 mg/kg. The therapeutic efficacy of conventional PZQ in the three doses was lower than orally administered PZQ-Si at corresponding doses about all histopathological parameters studied, and still there is a heavy lymphocytic infiltration that extends to the portal tract especially in the 250 mg/kg conventional PZQ-treated group compared to 250 mg/kg PZQ-Si (Table 5).

Measurement of different markers in positive control group indicated evident infection induced hepatic oxidative stress compared to the non-infected, non-treated control (Table 6). Dose-dependent enhancement of the antioxidant effect of PZQ was evident when it was encapsulated in MSNPs and orally administered in doses 250, 500, and 1000 mg/kg compared to positive control and conventional PZQ in 250, 500, and 1000 mg/kg, respectively.

Serum levels of IL-10 and TNF-α were significantly ($p \leq 0.05$) increased in the positive control mice compared to the non-infected, non-treated control (Table 7). Treatment with

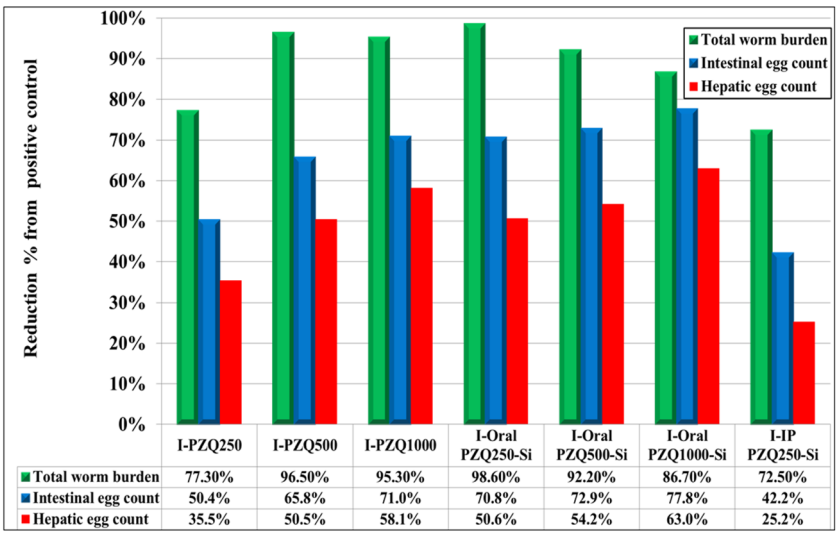

Fig. 4 Percent reduction of total worm burden and intestinal and hepatic ova counts from infected positive control group

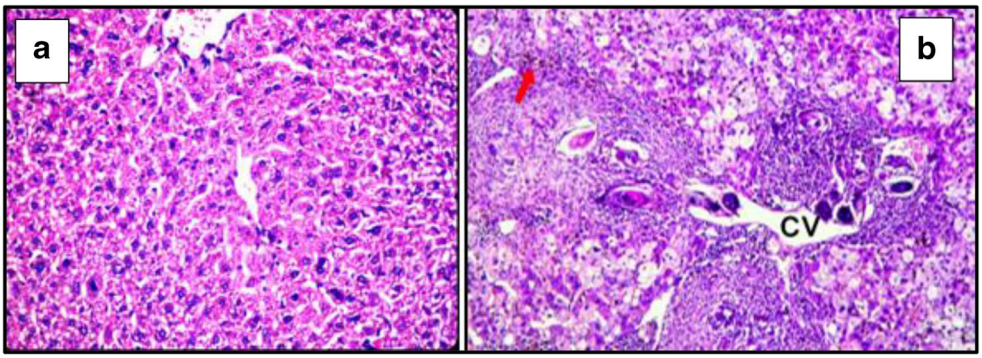

Fig. 5 (Hx and E stain ×400) (**a**) Liver tissue section of non-infected non-treated control group (N-N) shows intact hepatocytes arranged in thin plates with no degeneration, apoptosis or cytoplasmic vacuolation. (**b**) Liver tissue section of infected non-treated control group (I-C) shows multiple ill-defined granulomas surrounding a dilated central vein (CV). The granulomas surround one or more recently deposited intact or partially degenerated *S. mansoni* ova. Dilated hepatic sinusoids and hypertrophied Kupffer cells containing bilharzial pigment (red arrow) are observed

conventional PZQ significantly ($p \leq 0.05$) increased IL-10 and decreased TNF-α serum levels. Oral administration of PZQ encapsulated in MSNPs in doses 250, 500, and 1000 significantly ($p \leq 0.05$) enhanced the therapeutic effect more than that obtained with conventional PZQ 250, 500, and 1000 mg/kg, respectively, as evident by serum immunomodulatory marker levels (Fig. 9).

A high significant ($p \leq 0.05$) DNA fragmentation level was quantified in hepatic tissues of the positive control mice potentially concurrent with inflammatory granulomatous and oxidative stress reactions (Table 8). However, conventional PZQ treatment in its maximum dose (1000 mg/kg) led to a significant ($p \leq 0.05$) increase in the hepatic DNA fragmentation, compared to the positive control; treatment of infected mice with PZQ 1000 mg/kg loaded in MSNPs resulted in a significant decrease in hepatic DNA fragmentation (Fig. 10).

Partially to well circumscribed fibrocellular granulomas with the mononuclear inflammatory cellular component mainly seen in the periphery of the granuloma. Granulomas formed in the high and full-dose PZQ-Si-treated groups show a more dominant fibrous component (panels **c** and **d**). One or more viable partially degenerated *S. mansoni* ova are seen in the center of the granuloma (arrows) in reduced dose PZQ-Si orally and intraperitoneally treated groups (panels **a** and **b**). The high and full-dose PZQ-Si-treated groups that have intact surrounding parenchyma with minimal inflammatory infiltration and preserved central vein are observed in all PZQ-Si-treated groups.

Discussion

In the present work, PZQ was efficiently loaded in MSNPs with a mean entrapment efficiency of 83 ± 0.27%. MSNPs have the privilege of a solid framework with porous structure and large surface area, which allows to accommodate large quantities of the active drug molecules (Vallet-Regí et al. 2018). PZQ could be successfully encapsulated with EE of 82% in PLGA NPs (Mainardes and Evangelista 2005), 90% in poly-methyl-methacrylate (PMMA) NPs (da Fonseca et al. 2013), and 86.6% in SLN (Mishra et al. 2014). Designed MSNPs showed positive surface charge which may be attributed to the presence of the amine group of the silica nanoparticles. This result agreed with Torabi et al. (2017) and Liberman et al. (2014). Previous literature had reported that positively charged NPs showed increased cellular uptake into target cells, an advantage that favors the enhancement of their efficiency as a drug delivery system (Ruttala and Ko 2014). This was further explained by Honary and Zahir (2013) who reported that positively charged NPs prefer to contact with the negatively charged sulfated proteoglycans of the cell membrane via their electrostatic interaction, favoring their internalization into target cells. ZP value of prepared PZQ-Si was in favor of endorsing the stability with a moderate to high degree, according to the classification drawn by Bhattacharjee (2016).

TEM micrographs showed uniform spherical non-aggregated MSNPs, a character that favors the stability of the prepared NP suspension. Mishra et al. (2014) approved

Table 2 Physico-chemical characteristics of blank and praziquantel-loaded mesoporous silica nanoparticles

Prepared NPs	EE (%)	Mean diameter through DLS (nm)	Zeta potential (mV)
MSNPs	—	100 ± 5	31.9 ± 2.5
PZQ-Si	83 ± 0.27	105 ± 6	30 ± 2.3

Results are expressed as (mean ± SD)

EE encapsulation efficiency, *nm* nanometer, *mV*, millivolt

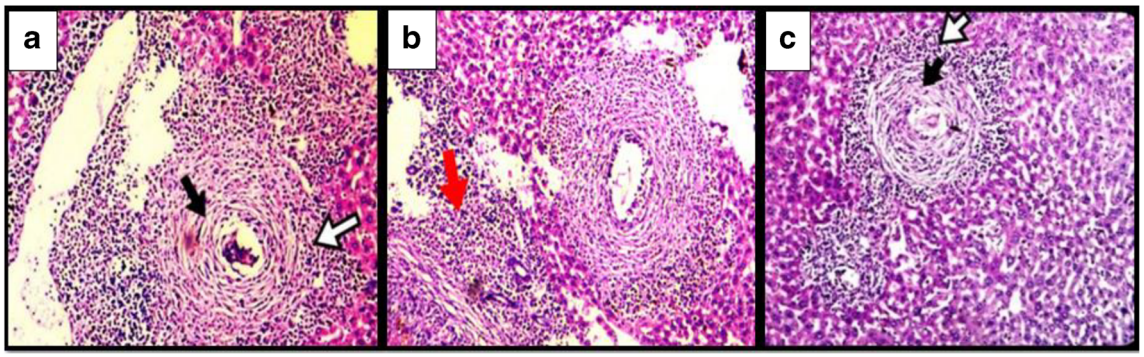

Fig. 6 Hepatic granulomas in infected group treated with conventional PZQ (I-PZQ). (**a**,**b**) I-PZQ_{250}; (**c**) I-PZQ_{1000} (Hx and E stain × 200). Partially circumscribed fibrocellular granulomas consisting of inflammatory cells with intervening collagen (black arrows) with the mononuclear inflammatory cells mainly are seen at the periphery of the granuloma (white arrows). The granuloma surrounds dead partially calcified or degenerated *S. mansoni* ova. Peri-granulomatous tissue in the reduced dose PZQ orally treated group (**b**) shows expansion of portal tracts by heavy lymphocytic infiltrate (red arrow). However, the intact peri-granulomatous tissue with marked reduction of inflammatory infiltration is observed in the full-dose PZQ orally treated group (**c**)

that spherical shape of NPs enhanced its high stability in refrigerated conditions for a six-month storage period. Spherical-shaped MSNPs show distinct endocytosis efficiencies into their target cells with better performance as drug delivery vehicles (Sun 2012). While, further particle size analysis through DLS showed that the average particle size of blank MSNPs was 100 ± 5 nm. The larger diameter as measured by DLS can be explained by the formation of the hydration film while NPs were dispersed in the dispersion medium during sample preparation for DLS (Zhao et al. 2017). DLS measurements showed an increase in size of MSNPs after loading with PZQ which indicates the successful incorporation of the PZQ into the nanocarrier system (Liu et al. 2016). Size range of the PZQ-Si was expected to effectively prolong the half-life of PZQ in the circulation in correlation to the fact that NPs around 100 nm exhibit enhanced circulation half-life compared to smaller or larger particles. Barua and Mitragotri (2014) reported that NPs smaller than 10 nm are extensively cleared by renal excretion. However, NP larger than 200 nm may encounter opsonization by plasma proteins (e.g., complement, immunoglobulins) in circulation and is common to develop hypersensitivity response (Sadat et al. 2016). Moreover, Gan et al. (2012) reported that the best internalization efficiency of MSNPs with the highest cellular uptake was shown by about 100-nm-sized particles. In addition, particle size in the present work was suitable for the parenteral route of administration as the diameter of the smallest blood capillaries is 4 μm (Lima et al. 2017).

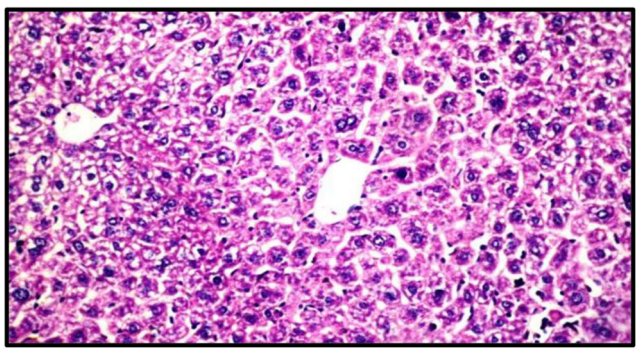

Fig. 7 Liver tissue section of non-infected mice treated with blank MSNPs (Hx and E stain × 400). No histopathological finding is observed

PZQ was administered either free or encapsulated in MSNPs in three different doses equally divided and given on two consecutive days. Xie et al. (2010) explained that splitting of the total PZQ dose into fractional doses enhances the efficacy over that achieved after a single oral administration of the same total dose. PZQ (1000 mg/kg) has been reported as the fully effective dose used for treatment of murine schistosomiasis *mansoni* (El-Beshbishi et al. 2013), while other experiments have studied PZQ (500 mg/kg) as a curative dose (El-Feky et al. 2015). The strategy from using half curative dose of PZQ (250 mg/kg) was that encapsulation in prepared NPs could protect the drug from rapid metabolism and clearance from the circulation and then improve its activity at even reduced dose. In the current study, encapsulation of PZQ in MSNPs in 250, 500, and 1000 mg/kg doses showed an enhanced antihelminthic effect compared to conventional PZQ 250, 500, and 1000 mg/kg, respectively, regarding parasitological and histopathological parameters. The reduced dose of 250 mg/kg of PZQ-Si decreased the total number of worms by 98.6%, the number of eggs in the intestine by 70.8% and in the liver by 50.6%, and the number and diameter of hepatic granulomas by 44.6 and 19.3% compared to 77.3, 50.4, 35.5, 40.8, and 12.8% by same reduced dose of conventional PZQ, respectively. This finding goes well with the results obtained in the in vivo experimental study done by Frezza et al. (2013) that the total worm burden and intestinal tissue egg load were significantly reduced by 34.8 and 61.8%, respectively, after oral administration of PZQ 250 mg/kg loaded in liposomal NPs and orally administered on the 45th day post-infection compared to 0 and 5.5%, respectively, by free PZQ. El-Feky et al. (2015) have prepared montmorillonite

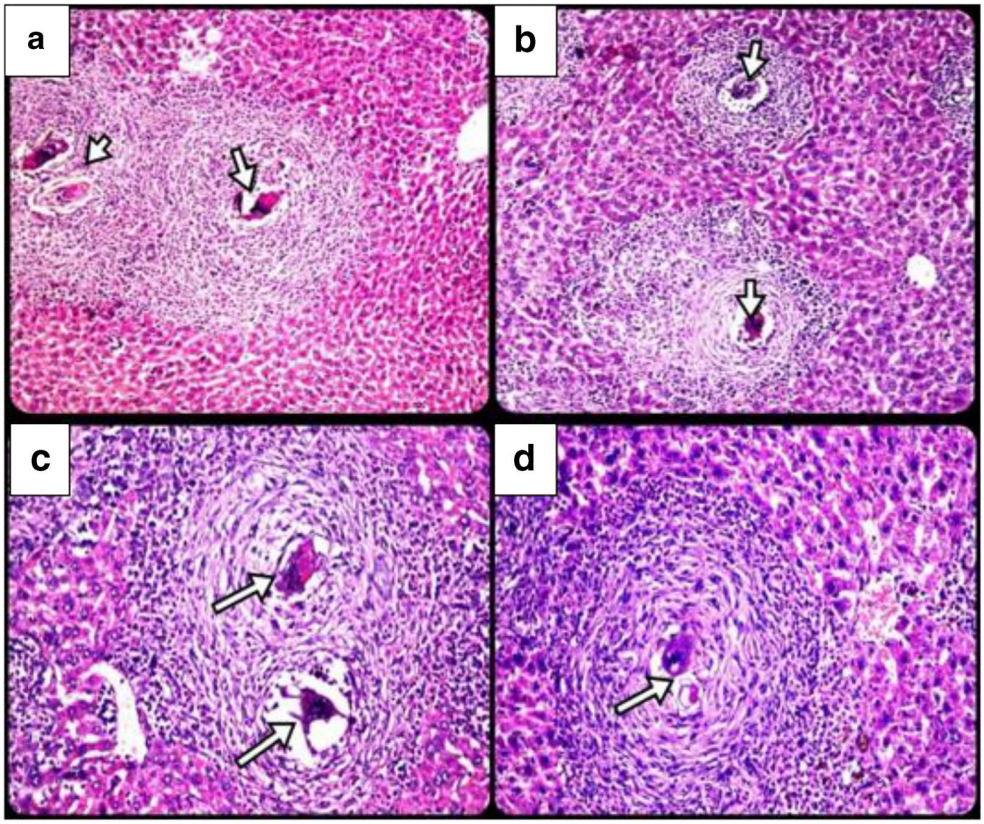

Fig. 8 Hepatic granulomas in infected group treated with PZQ-Si (I-PZQ-Si). (**a**) I-Oral PZQ250-Si (Hx and E stain × 200), (**b**) I-IP PZQ250-Si (Hx and E stain × 200), (**c**) I-Oral PZQ500-Si (Hx and E stain × 400), (**d**) I-Oral PZQ1000-Si (Hx and E stain × 400)

(MMT) clay NPs loaded with PZQ in doses of 250, 500, and 1000 mg/kg with enhanced antischistosomal activity compared to 250, 500, and 1000 mg/kg conventional PZQ therapy, respectively. Same finding was obtained with Amara et al. (2018) who recorded the ability of orally administered PZQ 250 mg/kg in lipid nanocapsules to reduce total worm burden

Table 3 Worm burden and intestinal and hepatic tissue egg load in *Schistosoma mansoni* infected mice treated with different doses of PZQ encapsulated in MSNPs 6 weeks post-infection (group B) compared with their corresponding controls (group A)

Animal groups (10 mice each)		Mean ± SD		
		Total worm burden	Intestinal ova count/gm tissue ($\times 10^3$)	Hepatic ova count/gm tissue ($\times 10^3$)
(A) Control groups	I-C	25.5 ± 7.4	30.5 ± 0.3	19.4 ± 0.1
	I-PZQ_{250}	5.8 ± 2.2	15.1 ± 0.6	12.5 ± 0.1
	I-PZQ_{500}	0.9 ± 0.9	10.4 ± 0.5	9.6 ± 0.1
	I-PZQ_{1000}	1.2 ± 1.9	8.8 ± 0.4	8.1 ± 0.2
(B) Study group	I-Oral PZQ_{250}-Si	0.4 ± 0.5[a]	8.9 ± 0.6[ab]	9.6 ± 0.1[a]
	I-Oral PZQ_{500}-Si	2.0 ± 1.2	8.3 ± 0.2[b]	8.9 ± 0.1[b]
	I-Oral PZQ_{1000}-Si	3.4 ± 1.1	6.8 ± 0.5[c]	7.2 ± 0.1[c]
	I-IP PZQ_{250}-Si	7.0 ± 1.2[d]	17.6 ± 0.5[d]	14.5 ± 0.1[d]
^P		≤ 0.001*		

^ANOVA test with post-hoc Tukey's HSD test

*Highly significant ($p \leq 0.001$)

All values were significant versus I-C

[a] Significant versus I-PZQ_{250}

[b] Significant versus I-PZQ_{500}

[c] Significant versus I-PZQ_{1000}

[d] Significant versus I-Oral PZQ_{250}-Si

Table 4 Oogram pattern in *Schistosoma mansoni*-infected mice treated with different doses of PZQ encapsulated in MSNPs 6 weeks post-infection (group B) compared with their corresponding controls (group A)

Animal groups (10 mice each)		Mean ± SD		
		Mature (%)	Immature (%)	Dead (%)
(A) Control groups	I-C	38.7 ± 0.6	57.2 ± 1.0	4.1 ± 0.9
	I-PZQ_{250}	39.5 ± 0.7**	17.6 ± 0.6	42.9 ± 0.6
	I-PZQ_{500}	41.6 ± 1.1	14.5 ± 0.7	43.9 ± 1.7
	I-PZQ_{1000}	28.4 ± 1.0	13.1 ± 0.8	58.5 ± 1.2
(B) Study groups	I-Oral PZQ_{250}-Si	36.9 ± 0.6[abc]	8.3 ± 0.6[abc]	54.8 ± 0.8[abc]
	I-Oral PZQ_{500}-Si	33.1 ± 0.7[b]	5.3 ± 0.8[b]	61.6 ± 1.3[b]
	I-Oral PZQ_{1000}-Si	35.2 ± 0.5[c]	5.2 ± 0.4[c]	59.6 ± 0.9
	I-IP PZQ_{250}-Si	15.7 ± 0.6[d]	38.1 ± 0.6[d]	46.2 ± 0.4[d]
^P		≤ 0.001*		

^ANOVA test with post-hoc Tukey's HSD test

*Highly significant ($p \leq 0.001$)

All values are significant versus I-C except **

[a] Significant versus I-PZQ_{250}

[b] Significant versus I-PZQ_{500}

[c] Significant versus I-PZQ_{1000}

[d] Significant versus I-Oral PZQ_{250}-Si

and mean granuloma number and diameter by 92.6, 60.3, and 54.1%, compared to 70.9, 37.0, and 32.2%, respectively, by 250 mg/kg conventional PZQ. Radwan et al. (2019) reported that compared to market PZQ (M-PZQ), parasitological findings revealed an increase in the percentage of *S. mansoni* worm reduction by 1.67-, 1.30-, and 1.06-fold in infected mice treated with PZQ encapsulated in solid lipid NPs (SLN-PZQ) at doses of 62.5, 125, and 250 mg/kg, respectively, in comparison to M-PZQ. El-Lakkany et al. (2011) mentioned that conventional PZQ 200 mg/kg failed to improve completely the hepatic pathological alterations induced by schistosomiasis. This was further strengthened by the work of Aboueldahab and Elhussieny (2016) who reported that the effect of orally administered PZQ 250 mg/kg on histopathological parameters was significantly lower than that of PZQ 250 mg/kg when combined with curcumin. Currently, the high

Table 5 Results of histopathological study of liver in infected mice treated with different doses of PZQ encapsulated in MSNPs 6 weeks post-infection (group B) compared with controls (group A)

Animal groups (10 mice each)		Mean ± SD		
		Number of granulomas in power fields (10 × 10)	Diameter of granuloma in (μm)	% of intact eggs in the granuloma
(A) Control group	I-C	10.5 ± 2.3	341.0 ± 35.7	95.0 ± 1.3
	I-PZQ_{250}	6.2 ± 1.2	297.5 ± 24.6	92.0 ± 2.4
	I-PZQ_{500}	5.9 ± 1.9	253.2 ± 34.6	90.0 ± 2.0
	I-PZQ_{1000}	5.5 ± 1.5	242.2 ± 28.8	85.0 ± 1.9
(B) Study group	I-Oral PZQ_{250}-Si	5.8 ± 1.2	275.1 ± 28.2	92.0 ± 2.9[b]
	I-Oral PZQ_{500}-Si	5.3 ± 1.3	221.0 ± 15.5	84.0 ± 1.5[a]
	I-Oral PZQ_{1000}-Si	5.0 ± 1.1	214.3 ± 19.2	84.0 ± 1.8
	I-IP PZQ_{250}-Si	6.2 ± 1.3	280.0 ± 21.8	95.0 ± 1.6[c]**
^P		≤ 0.001*		

^ANOVA test with post-hoc Tukey's HSD test

*Highly significant ($p \leq 0.001$)

All values are significant versus I-C except **

[a] Significant versus I-PZQ_{500}

[b] Significant versus I-PZQ_{1000}

[c] Significant versus I-Oral PZQ_{250}-Si

Table 6 Oxidative stress markers; reduced glutathione (GSH), malondialdehyde (MDA), and nitric oxide (NO) levels in liver tissue homogenate in *Schistosoma mansoni*-infected mice treated with different doses of PZQ encapsulated in MSNPs 6 weeks post-infection (group B) compared with their corresponding controls (group A)

Animal groups (10 mice each)		(Mean ± SD)		
		GSH (mg/gm)	MDA (nmol/gm)	NO (μmol/gm)
(A) Control group	N-N	97.7 ± 2.0	44.7 ± 0.1	14.0 ± 1.3
	I-C	42.7 ± 6.5	65.5 ± 0.1	25.0 ± 6.1
	I-PZQ_{250}	45.6 ± 0.2	65.2 ± 0.1	24.9 ± 1.4
	I-PZQ_{500}	48.8 ± 1.9	64.4 ± 0.1	24.5 ± 0.1
	I-PZQ_{1000}	50.9 ± 7.9[a]	61.5 ± 0.1[a]	21.9 ± 0.4[a]
(B) Study group	I-Oral PZQ_{250}-Si	64.8 ± 0.1[abcd]	54.3 ± 0.1[abcd]	19.0 ± 0.2[abcd]
	I-Oral PZQ_{500}-Si	79.8 ± 0.4[ac]	50.6 ± 0.0[ac]	16.6 ± 0.2[ac]
	I-Oral PZQ_{1000}-Si	84.4 ± 6.2[ad]	46.8 ± 2.7[ad]	15.6 ± 0.9[a]d**
	I-IP PZQ_{250}-Si	64.3 ± 0.4[a]	57.3 ± 0.5[ae]	19.9 ± 0.0[a]
^P		≤ 0.001*		

^ANOVA test with post-hoc Tukey's HSD test

*Highly significant ($p \leq 0.001$)

All values were significant versus N-N except **

[a] Significant versus I-C

[b] Significant versus I-PZQ_{250}

[c] Significant versus I-PZQ_{500}

[d] Significant versus I-PZQ_{1000}

[e] Significant versus I-Oral PZQ_{250}-Si

therapeutic efficacy observed by PZQ-Si was also in context with the proved efficiency of MSNPs as a controlled delivery system for many hydrophobic drugs like benznidazole with remarkable in vitro growth inhibition of *T. cruzi* (Nhavene et al. 2018) and antimalarial drug, quinine, with promising results (Amolegbe et al. 2018).

Schistosomiasis-induced hepatic oxidative stress in the positive control group is explained by previous reports. During schistosome infection, macrophages produce NO and reactive oxygen species (ROS) (Hassan et al. 2016). ROS that are released result in the increase of toxic oxygen radicals, hydrogen peroxide, and hydroxyl radicals, with subsequent increase of lipid peroxidation. MDA is the end product of lipid peroxidation in the liver (Kadry et al. 2013). With schistosomiasis, there is impairment of liver GSH content with the decrease of the antioxidant capacity of the liver (Fahmy et al. 2014). In the present study, treatment of infected mice by 250, 500, and 1000 mg/kg conventional PZQ was associated with improvement of the hepatic oxidative stress status which was significant only in the full-dose PZQ-treated group. This was in acceptance with Jatsa et al. (2015) who reported the failure of conventional PZQ to correct the disturbed levels of oxidative stress biomarkers in schistosomiasis-infected mice. Currently, dose-dependent enhancement of the antioxidant effect of PZQ was evident when it was encapsulated in MSNPs and orally administered in its three different doses compared to conventional PZQ in the same doses. Comparing GSH, MDA and NO levels in the infected groups treated with oral PZQ-Si 250 mg/kg with group receiving conventional PZQ 250 mg/kg showed a statistically significant ($p \leq 0.05$) difference, with higher levels of GSH and lower levels of MDA and NO in the former group. This could be related to the significant reduction of the parasite load achieved by treatment and to the fact that macrophages and especially the liver-resident Kupffer cells show highly efficient and unspecific uptake of most nanomaterials. This can suggest the passive targeting of nanotherapeutics to the liver with their enhanced performance in chronic liver diseases (Bartneck et al. 2014). MSNPs have been reported to exhibit a strong antioxidant activity, a property that may be of value when encapsulating PZQ (Morry et al. 2017). On contrary, this disagrees with Chen et al. (2018) who reported that MSNPs increase the release of lipid peroxides and reduce antioxidant enzyme activities in the liver after repeated intravenous exposure. Discrepancy between studies could be related to difference in the physico-chemical properties of the prepared MSNPs as well as doses and routes of administration.

The increased serum levels of IL-10 and TNF-α in the positive control mice were explained previously. El-Sayed et al. (2016) illustrated that TNF-α is secreted by activated macrophages in response to parasitic soluble antigens and reported that macrophages stimulate Th1 cells to secrete more TNF-α. IL10 reduces the acute pathology of schistosomiasis; being essential for the generation of host protective homeostatic conditions to maintain a non-lethal chronic infection (Farrag et al. 2015). In both groups, conventional PZQ and PZQ-Si treated mice, reported decreased TNF-α and increased IL-10 serum levels collaborate well with the

parasitological and histopathological findings. This was further supported by Aly et al. (2010) who claimed that the increase in IL-10 with PZQ treatment could reduce the granuloma size. El-Sayed et al. (2016) reported that TNF-α serum level was significantly reduced in PZQ-treated mice compared to positive control. Comparing serum levels of TNF-α and IL-10 in infected groups treated with oral PZQ-Si 250, 500, and 1000 mg/kg with groups receiving conventional PZQ 250, 500, and 1000 mg/kg, respectively, showed a statistically significant ($p \leq 0.05$) difference. Previous results in literature have demonstrated that MSNPs are incapable of inducing specific immune responses when used blank (unloaded) (Carvalho et al. 2010; Wang et al. 2013). So, the more immunomodulatory effect observed in groups treated with PZQ-Si can be attributed to the higher bioavailability of PZQ released from PZQ-Si compared to free PZQ. Oliveira et al. (2016) studied MSNPs as a potential SWAP vaccine adjuvant against *Schistosoma mansoni*, and their immune assays indicated that SWAP-loaded MSNPs are capable of stimulating higher immune response, in comparison to conventional adjuvants.

When PZQ was encapsulated in MSNPs at a reduced dose 250 mg/kg, it showed an enhanced significant antischistosomal effect compared to conventional PZQ at a higher dose 500 mg/kg. This reduced dose of PZQ-Si resulted in a decrease in intestinal tissue egg count coupled with a decrease in mature and immature eggs and increase in dead eggs. Moreover, it resulted in healing of hepatic granulomas with disappearance of active cellular granuloma as similarly observed on administration of conventional PZQ in high and full doses. Furthermore, treatment of infected mice with reduced dose of PZQ-Si significantly ($p \leq 0.05$) modulated the oxidative stress markers and resulted in a significant ($p \leq 0.05$) elevation of IL-10 and reduction of TNF-α compared to the profile observed in the groups treated with high and full doses of conventional PZQ. This further supports the significant increase in the therapeutic efficiency of PZQ even in a reduced dose (250 mg/kg) when loaded into MSNPs. The human dose equivalent can be more appropriately calculated by using the formula mentioned by Reagan-Shaw et al. (2008).

In the present study, PZQ-Si was given by parenteral administration in order to achieve a direct systemic delivery of PZQ thereby avoiding first-pass hepatic metabolism and improving its therapeutic efficacy (Frezza et al. 2013). Free PZQ is administered to humans only by the oral route; however, oral drugs display higher variability than drugs administered by the parenteral route, which is explained by the blood flow at the absorption site, the absorptive surface area, the transit time, and the gastric pH. These factors are also influenced by concurrent food intake; the bioavailability of PZQ increases with a fatty diet and a high-carbohydrate diet administration. In addition, PZQ binds to proteins; hence, nutritional status and other factors, including chronic inflammation, influence the levels of the free drug orally administered (Vale et al. 2017). Currently, 250 mg/kg PZQ-Si intraperitoneally administered demonstrated a lower antischistosomal efficiency in comparison to 250 mg/kg PZQ-Si when orally administered considering studied parasitological and histopathological parameters as well as in oxidative stress and immunological studies. The current findings however contrasted with Mainardes and Evangelista's (2005) study that recommended

Table 7 Serum levels of interleukin-10 (IL-10) and tumor necrosis factor-alpha (TNF-α) in *Schistosoma mansoni*-infected mice treated with different doses of PZQ encapsulated in MSNPs 6 weeks post-infection (group B) compared with their corresponding controls (group A)

Animal groups (10 mice each)		(Mean ± SD)	
		Cytokines serum level in (pg/ml)	
		IL-10	TNF-α
(A) Control group	N-N	175.6 ± 2.6	91.7 ± 0.3
	I-C	305.2 ± 2.3	664.6 ± 0.9
	I-PZQ_{250}	385.3 ± 0.9	412.6 ± 0.9
	I-PZQ_{500}	384.3 ± 0.8	585.5 ± 0.8
	I-PZQ_{1000}	488.5 ± 1.8	456.2 ± 0.3
(B) Study group	I-Oral PZQ_{250}-Si	681.4 ± 1.3[abc]	305.8 ± 0.6[abc]
	I-Oral PZQ_{500}-Si	783.0 ± 1.4[b]	294.1 ± 0.3[b]
	I-Oral PZQ_{1000}-Si	820.7 ± 1.0[c]	263.5 ± 0.5[c]
	I-IP PZQ_{250}-Si	591.8 ± 1.0[d]	372.3 ± 0.6[d]
^P		≤0.001*	

^ANOVA test with post-hoc Tukey's HSD test

*Highly significant ($p \leq 0.001$)

All values were significant versus I-C and N-N

[a] Significant versus I-PZQ_{250}

[b] Significant versus I-PZQ_{500}

[c] Significant versus I-PZQ_{1000}

[d] Significant versus I-Oral PZQ_{250}-Si

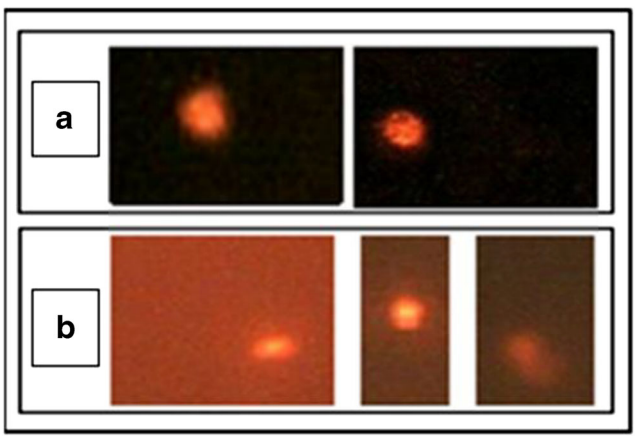

Fig. 9 Comet assay results in liver tissue of non-infected mice two weeks post treatment with blank MSNPs (**b**) compared with negative control group (**a**)

Table 8 Assessment of DNA damage in liver tissue of both non-infected mice treated with blank MSNPs and infected mice treated with full dose of PZQ either free or encapsulated in MSNPs 2 weeks post-treatment, compared with positive and negative control groups

Animal group (10 mice each)	Mean ± SD	
	Tail intensity (% of DNA in tail)	Tail moment (arbitrary unit)
N-N	1.13 ± 0.32	0.22 ± 0.05
N-Si	1.61 ± 0.21	0.29 ± 0.06
I-C	4.01 ± 0.10[a]	0.64 ± 0.03[a]
I-Oral PZQ_{1000}	5.04 ± 0.13[b]	0.78 ± 0.04[b]
I-Oral PZQ_{1000}-Si	3.32 ± 0.11[bc]	0.41 ± 0.02[bc]
^P	< 0.001*	

^ANOVA test with post-hoc Tukey's HSD test

*Highly significant ($p \leq 0.001$)

[a] Significant against N-N

[b] Significant against I-C

[c] Significant against I-Oral PZQ_{1000}

the use of intravenous route to improve PZQ bioavailability when loaded in PLGA nanoparticles. Furthermore, Xie et al. (2010) demonstrated that PZQ-loaded in hydrogenated castor oil (HCO) solid lipid nanoparticles (SLN), delivered by subcutaneous and intramuscular routes, greatly improved the bioavailability and extended the systemic circulation of PZQ. Discrepancy in current results may be attributed to the limited distribution of PZQ-Si after intraperitoneal injection (Xie et al. 2010). Furthermore, Fu et al. (2016) reported that IP administration of a chemotherapeutic drug encapsulated in MSNPs was associated with the residence of the drug in the peritoneum for a longer time with the reduction of the systemic exposure to the administered drug that did not have direct access to the circulation.

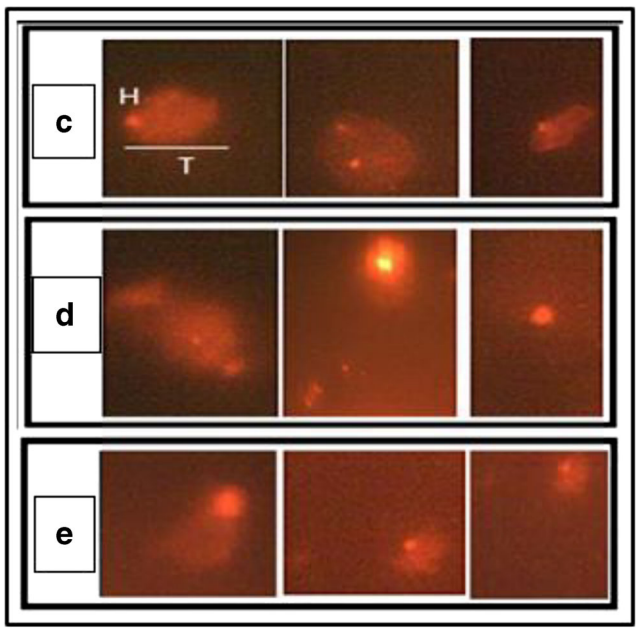

Fig. 10 Comet assay results in liver tissue of infected mice two weeks post-treatment with full doses of conventional PZQ (**d**), PZQ-Si (**e**) compared with positive control group (**c**)

Currently, the enhancement of antischistosomal *mansoni* efficacy of praziquantel by incorporating into MSNPs may be correlated to improvement of PZQ bioavailability after oral administration. It was reported that NPs, due to their small particle size, enable strong bioadhesion of the nanoformulation to the gastrointestinal tract wall with increased residence time. Moreover, small-sized NPs contribute to increased permeability of the intestinal membrane (Xie et al. 2010). More specifically, MSNPs can be efficiently internalized by a variety of mammalian cells, enhancing the delivery of the encapsulated drug into target cells with the increase of its therapeutic efficiency (Shang et al. 2014). PZQ was proved through in vivo experiments to not only affect the tegument and muscular structure of the worm but also act on vitelline cells and ovaries (Frezza et al. 2013). Therefore, the evident decrease in oviposition as noted in the present work may be due to greater uptake of PZQ when encapsulated in MSNPs by ovaries. Furthermore, the MSNPs can exert a better control over the drug diffusion kinetics allowing more sustained drug release (Vallet-Regí et al. 2018). The antischistosomal effect of PZQ relates not only to the absolute height of the maximal plasma concentration but also to the length of exposure to the drug (Xie et al. 2010).

In the current study, comet assay was selected for determination of hepatic DNA damage owing to its distinct advantage that both single- and double-stranded breaks in damaged DNA can be detected at a low cost with high sensitivity (Barnes et al. 2008). The observed hepatic DNA fragmentation in positive and PZQ (1000 mg/kg) control groups agreed with previous reports that *Schistosoma mansoni* infection-induced oxidative stress leads to fragmentation of nuclear DNA in the liver, which contributes to hepatocellular apoptosis as well as necrosis (Mukhopadhyay et al. 2011). Omar et al. (2005) observed that PZQ induced a significant increase in the incidence of chromosomal aberrations as fragmentation, deletion, and ring chromosome formation concluding that

PZQ shows a hepatotoxic and genotoxic potential. Currently, the non-toxic nature of MSNPs agreed with Johnston et al. (2000) who denied the induction of any genetic mutation in rats after 13 weeks of exposure to MSNPs. On the other hand, Choi et al. (2011) reported that MSNPs can cause primary DNA damage but not mutagenicity in cultured mammalian cells (mouse lymphoma cell line and human bronchial epithelial cells). Contradiction between the results of different studies could be related to different methods and protocols used for assessment of the genotoxicity, in addition to different types of cells used (Barnes et al. 2008).

In conclusion, encapsulation of PZQ in MSNPs improved the efficiency of PZQ on one hand. On the other hand, a reduction of the therapeutic dose of PZQ to be 250 mg/kg is recommended, an advantage that may add to lowering the side effects of conventional PZQ treatment. Data obtained implied that IP route is less efficacious for the delivery of PZQ-Si. Further studies of in vitro PZQ release and in vivo PZQ bioavailability as well as efficacy against *S. mansoni* larval stages are recommended using PZQ-Si compared to conventional PZQ. Analysis of the cost and physical stability of Si-PZQ after variable storage periods should be also taken into consideration for future clinical use. The toxicity, direct effect on worm, and all possible immunological properties of unloaded and PZQ loaded MSNPs on infected and uninfected mice should be evaluated more rigorously to confirm the accuracy of the data for extensive use.

Compliance with ethical standards

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

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

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