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Research Article
Evaluation of Solid Lipid Nanoparticles Loaded with Praziquantel for Treatment of *Schistosoma mansoni* Infected Rats

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Abstract

Background and Objectives: Solid Lipid Nanoparticles (SLN) are a promising drug delivery system for oral administration of poorly water soluble drugs. Solid Lipid Nanoparticles (SLN) are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery and research. The present work aimed to evaluate the efficacy of SLN loaded with praziquantel (PZQ) for treatment of schistosomiasis. **Materials and Methods:** The SLN was prepared and characterized to be a carrier for PZQ. Seventy male rats were divided equally into 7 groups (10 rats/group); normal control, infected control, infected and received SLN at 14 and 35 days, infected and received SLN loaded with PZQ which administered orally as two consecutive doses at days 14 and 35 post-infection, the last group infected and received PZQ. All rats were sacrificed after 8 weeks PI. **Results:** Liver section showed that treatment with SLN-PZQ the granuloma diameter was reduced. ALT and AST levels were decreased. Total Protein (TP) was increased and Gamma-glutamyl transferase was decreased. Level of IL-4 was decreased while, IL-10 level was high in treated group. Administration of PZQ at 35 days showed complete disappearance of ova. However, administration of SLN loaded with PZQ at 35 days post-infection decreased the worm burden and ova count in both liver and intestine compared to infected untreated group. **Conclusion:** Results of the present study conclude that the encapsulation of PZQ in SLN improved the safety profile of the drug. The PZQ in SLN could be a promising formulation to enhance the pharmacological activity of PZQ.

Key words: Solid lipid nanoparticles, *Schistosoma mansoni*, curative drug, histopathological studies, liver enzymes, cytokine level

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Schistosomiasis is "an acute and chronic parasitic disease caused by blood flukes (trematode worms) of the genus *Schistosoma*[1-3]. It is a great public health problem in 78 countries, particularly tropical and subtropical regions. It is estimated that 92% of patients live in Africa[4]. The disease causes health and labor loss and finally a significant reduction in socioeconomic benefits. Egypt has been plagued by schistosomiasis and it was traditionally the most important public health problem[5]. Intestinal schistosomiasis can result in abdominal pain, diarrhoea and blood in the stool. Liver enlargement is common in advanced cases and is frequently associated with an accumulation of fluid in the peritoneal cavity and hypertension of the abdominal blood vessels. In such cases there may also be enlargement of the spleen[6]. There are no symptoms when first infected. After few days of infection, patients could develop a rash or itchy skin. After 1-2 months of infection, symptoms may develop including fever, chills, cough and muscle aches[7]. Preventive treatment must be repeated for several years to help in reducing and preventing morbidity. This preventive chemotherapy treatment is only required in 52 endemic countries with moderate to high transmission[3]. Treatment of schistosomiasis serves three purposes; reversing acute disease, preventing complications[6] and preventing neuroschistosomiasis[8,9]. Due to its poor solubility in water, low oral bioavailability and risk of parasite resistance to praziquantel, it would be useful to develop a novel product that can overcome these shortcomings by making praziquantel an excellent candidate for encapsulation in solid lipid nanoparticles[10,11]. Solid lipid nanoparticles combine the advantages of different colloidal carriers and also avoid some of their disadvantages[12-14]. Nanotechnology is the study of controlling matter on an atomic and molecular scale to create materials and devices with specific properties. Nanotechnology is the dealing of individual atoms, molecules or compounds into structures to create materials and devices with specific properties[15]. Solid Lipid Nanoparticles (SLN) are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery and research[16]. The SLNs combine numerous advantages over the other colloidal carriers i.e., incorporation of lipophilic and hydrophilic drugs feasible, no bio-toxicity of the carrier, avoidance of organic solvents, possibility of controlled drug release and drug targeting, increased drug stability and no problems with respect to large scale production[17]. The present work was aimed to examine the parasitological parameters and histopathological changes of infected rats compared to normal and SLN-PZQ treated rats.

MATERIALS AND METHODS

Preparation and characterization of nanoparticles-loaded PZQ (SLN-PZQ): Formulation of SLN contained 10% of hydrogenated palm oil and hydrogenated lecithin was performed according to Liedtke *et al.*[18]. All components had been weighted into sealed containers and heated to 80°C. Thereafter, a preemulsion used to be produced using an Ultra Turrax (Ika/Staufen, Germany) at 10,000 rpm for 10 min. The warm pre-emulsions had been ecrystalli at a strain of 1000 bar and a temperature of about 70°C with an Emulsi Flexw-C5 (Avestin, Canada) high strain re-crystallized for 10 cycle. Subsequently, the dispersions were allowed to re-crystallize at room temperature[18]. The average diameter and Polydispersity Index (PI) of SLN were determined by Photon Correlation Spectroscopy (PCS) by using a Zetasizer (Malvern Instruments, Malvern, UK) at a fixed angle of 90 and at 25°C.

PZQ-loaded nanoparticles were formed by the addition of SLN to 10% of hydrogenated palm oil Softisan 154 and hydrogenated lecithin (lipid matrix), 1% oleyl alcohol, 0.005% thimerosal and 89% bidistilled water (all w/w) with 500 mg mL^{-1} concentrations of PZQ.

Experimental design: Study was carried out between June, 2018-December, 2019 in Parasitology Department at Theodore Bilharz Research Institute (TBRI) Giza, Egypt. Seventy male Swiss Webster rats, weighing 70 g, age 6 weeks will be used in all experiments. The animals will be divided randomly into 7 groups (control and infected groups) of 10 animals each.

All infected groups subcutaneously receive approximately 100 cercariae of *S. mansoni*. The subcutaneous injections will be administered according to the technique described by Peters and Warren[19]:

Group 1: Normal control group received normal diet (non-infected non-treated)
Group 2: Infected and received normal diet (infected non-treated)
Group 3: Infected with *S. mansoni* and received SLN (50 μg kg^{-1}) for two consecutive days at 14 days after infection
Group 4: Infected with *S. mansoni* and received SLN (50 μg kg^{-1}) loaded with PZQ (500 mg kg^{-1}) for two consecutive days at 14 days after infection
Group 5: Infected with *S. mansoni* and received SLN (50 μg kg^{-1}) for two consecutive days at 35 days after infection

Group 6: Infected with *S. mansoni* and received SLN (50 µg kg^{-1}) loaded with PZQ (500 mg kg^{-1}) for two consecutive days at 35 days after infection

Group 7: Infected with *S. mansoni* and received two doses of PZQ (500 mg kg^{-1}) for two consecutive days at 35 days after infection

Rats of all experimental groups were sacrificed at 8 weeks and were subjected to the following investigations.

Serum preparation: Blood samples collected in centrifuge tubes were centrifuged at 3000 rpm for 20 min. Serum was stored at 20°C until used for biochemical assays.

Tissue homogenate preparation: Liver tissue was homogenized (10% w/v) in ice-cold 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm for 15 min at 4°C and the resultant supernatant was used for biochemical analysis.

Assessment of biochemical parameters: The Biodiagnostic kits (Dokki, Giza, Egypt) were used for the determination of serum aminotransferase enzymes (AST and ALT) activities[20], alkaline phosphatase (ALP)[21] and total protein[22]. Spectrum kit (Obour, Cairo, Egypt) was used for the determination of Gamma-Glutamyl Transferase (GGT)[23].

Parasitological criteria

Worm burden: Adult worms were harvested by hepatic and intestinal perfusion 8 weeks after infection according to the method described by Duvall and DeWitt[24].

Tissue egg load (liver and intestine): The number of eggs per gram tissue (liver and intestine) were studied according to the procedure described by Cheever[25].

Percentage egg developmental stages "Oogram Pattern": The percentages of immature, mature and dead ova in the small intestines were computed from a total of 100 eggs per intestinal segment and classified according to the categories previously defined by Pellegrino *et al.*[26].

Histopathological examination

Preservation and processing: Liver portions from each animal was removed and fixed in 10% buffered formalin solution. Then processed in pathology lab and embedded in paraffin wax to be sectioned and stained with hematoxylin and eosin stain for routine histopathological examination.

Determination of oxidative stress markers

Determination of nitric oxide content in liver and kidney homogenate: Nitric Oxide (NO) is a simple, inorganic, gaseous free radical which is an important bioactive agent and a signaling molecule and may contribute to the pathogenesis of cancer[27].

Measurement of IL-10 and IL-4 in serum of infected and control groups: Serum IL-10 concentration was determined in serum following the company protocol "Abcam's KITab108870-IL-10 (Interleukin-10) Rat ELISA Kit-Cambridge, UK.

Serum IL-4 concentration was determined in serum following the company protocol "Abcam's KITab100770-IL-4 (Interleukin-4) Rat ELISA Kit-Cambridge, UK".

Statistical analysis: Data were presented as mean±SD. Group means and standard deviations for all groups were calculated and compared. Statistical analysis will be assessed by Student's t-test and one way ANOVA at p-value<0.05 to test whether there is significant difference between praziquantel and its nanoparticle formula.

RESULTS

Parasitological parameters: The worm burden and tissue egg load in the intestine and liver were calculated for each studied group (Table 1 and Fig. 1). In the infected control group, the total number of worms counted was 26±2.9. Oral administration of SLN to mice at day 14 or 35 PI reduced the total worm burden to 21.2±1.16 (8.5% reduction) and 13±0.8 (50%) whereas, oral administration of SLN loaded with PZQ to mice at day 14 or 35 PI reduced the total worm burden to 9.5±1.1 (63.5% reduction) and 1.2±1.9 (93.4%). The PZQ treatment (500 mg kg^{-1}) at late PI reduced the total worm burden to 1.7±0.15 (92.5% reduction) (Fig. 1). The egg load both in the intestine and in the liver were reduced in

Table 1: Worm burden of the studied groups treated with solid lipid nanoparticles alone or loaded with praziquantel

	Worm burden			
Groups	Mean of worms	SD	Reduction (%)	p-value
Infected control	26.0	2.90	-	-
SLN early	21.2	1.16	8.50	NS
SLN early+PZQ	9.5	1.10	63.50	**
SLN late	13.0	0.80	50.00	*
SLN late+PZQ	1.2	0.19	93.40	***
PZQ late	1.7	0.15	92.50	***

NS: Non-significant, SD: Standard deviation, *.**.***Significantly decreased than infected (p<0.05, p<0.001, p<0.001, respectively)

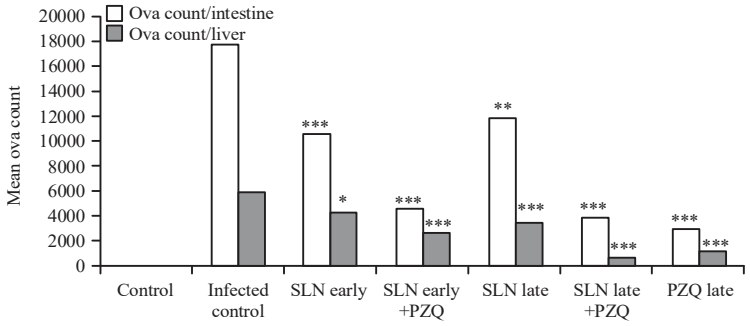

Fig. 1: Effect of treatment with solid lipid nanoparticles alone or loaded with praziquantel on tissue egg load of *S. mansoni* infected rats

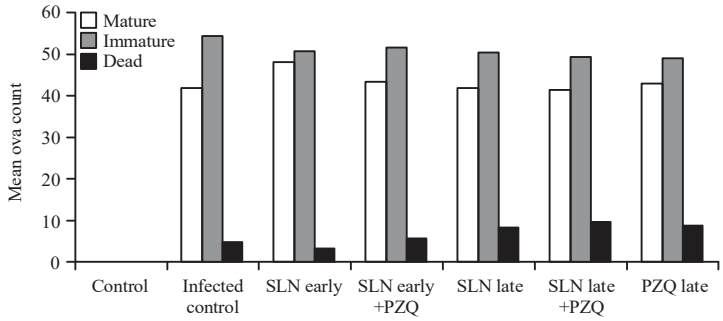

Fig. 2: Effect of treatment with solid lipid nanoparticles alone or loaded with praziquantel on tissue on organ pattern of *S. mansoni* infected rats

Table 2: Serum levels of serum biochemical activities in studied groups treated with SLN alone or loaded with PZQ

Groups	ALT	AST	TP	GGT	AFP
Normal control	49.1±3.03	41.7±1.76	6.91±0.56	3.7±0.22	6.20±0.04
Infected control	74.3±5.25	68.0±2.66	8.07±0.49	5.4±0.11	7.60±0.07
SLN early	44.5±3.68	46.9±2.51	7.27±0.70	5.1±0.34	7.10±0.06
SLN early+PZQ	61.3±3.46	58.7±3.65	7.82±0.13	4.9±0.43	6.96±0.07
SLN late	54.1±4.72	51.0±2.16	7.71±0.33	5.0±0.32	7.00±0.04
SLN late+PZQ	54.0±3.82	50.2±1.54	7.40±0.34	3.9±0.43	6.50±0.05
PZQ late	45.9±1.96	41.3±1.33	7.58±0.26	4.1±0.16	6.61±0.06

AST and ALT: Serum aminotransferase enzymes, ALP: Alkaline phosphatase, TP: Total protein, GGT: Gamma-glutamyl transferase

accordance to the worm burden (Fig. 1). Following SLN loaded with PZQ treatment to mice at day 14 or 35 PI, the oogram pattern showed increased in dead ova in group that treated with SLN loaded with PZQ (Fig. 2).

Biochemical parameters: As shown in Table 2, the levels of serum ALT, AST, AFP and GGT activities were significantly increased in the serum of *S. mansoni* infected mice as compared to normal control. Treatment of rats with SLN alone or loaded with PZQ either early at or late PI significantly decreased the activities of studied enzymes as compared to infected untreated group. The best reduction of the activities of studied enzymes was observed in group treated with SLNP loaded with PZQ at 35 PI. Data recorded in Table 2 showed decreased in the serum total protein and GGT concentration following *S. mansoni* infection as compared to control group. In comparison with infected untreated control, serum total protein concentration of rats administered SLNP loaded with PZQ at 35 PI revealed increased level.

Determination of nitric oxide content in liver and kidney homogenate: Figure 3a-b shows level of NO in kidney and liver homogenate (μmol g^{-1} tissue used) of the studied groups. Mean NO kidney was highest among SLN late and lowest among control then SLN-PZQ late treated group. Mean NO liver was highest among infected control and lowest among control then SLN-PZQ late treated group, F-test shows statistical significance at $p<0.001$.

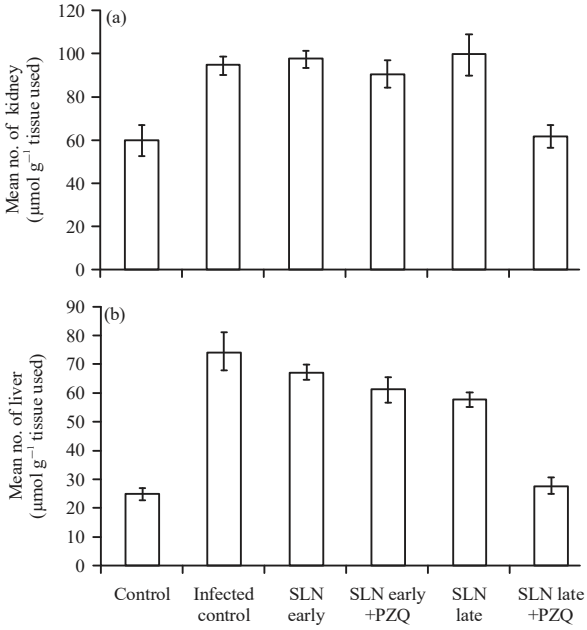

Fig. 3(a-b): Level of number in (a) Kidney and (b) Liver tissue of the studied groups

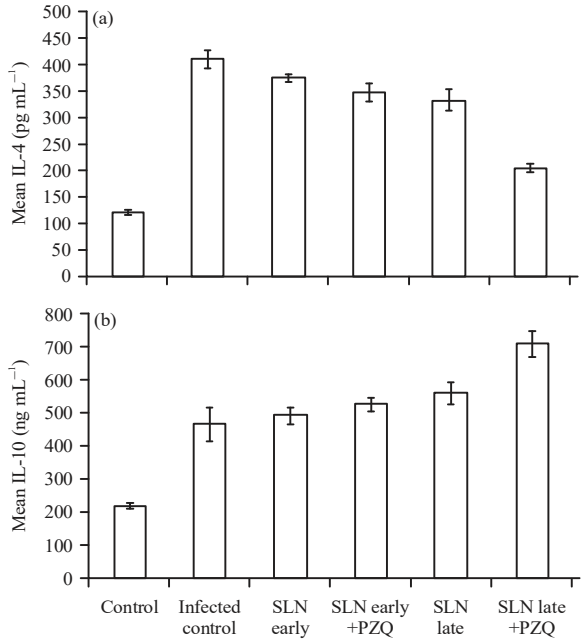

Fig. 4(a-b): Level of (a) IL-4 and (b) IL-10 in serum of the studied groups

Determination of immunological parameters using Enzyme Linked Immuno Sorbent Assay (ELISA): Figure 4a-b shows level of IL-4 (pg mL^{-1}) and IL-10 (ng mL^{-1}) of the studied group. Mean IL-4 is highest among infected control and lowest among control then SLN-PZQ late treated group. Mean IL-10 is highest among SLN late+PZQ then PZQ late treated group and lowest among control then infected control, F-test shows statistical significance at $p<0.001$.

DISCUSSION

Schistosomiasis is an important tropical disease, endemic in 76 countries, that afflicts more than 240 million people[28]. There is no vaccine for schistosomiasis and chemotherapy depends closely on a single drug, praziquantel[29], even though PZQ is a very efficacious and safe antischistosomal drug. It has some disadvantages, as stage-dependent susceptibility and terrible efficacy towards immature schistosome stages[30]. Therefore, there is an urgent need to develop a new antischistosomal drug. Nanoparticles have received more attention as antiparasitic drugs in recent years since current antiparasitic drugs have some side effects and their efficacy is not proved yet[31]. The treatment was recommended in several studies as it provided many complementary goals, a reduction of egg-induced pathology, minimal parenchymal changes and the eradication of worms[32]. Previous studies focused their studies on the epidemiology of schistosomiasis or the physiology of the parasites neglecting to some extent the metabolic changes developed in the host in consequence to infection or PZQ treatment. Therefore, the assessment of nanoparticles loaded with PZQ treatment efficacy in infected rats is important for the evaluation of the magnitude of infection and efficacy of the treatment. The present study showed that the administration of SLNP loaded with PZQ at 14 days or late at 35 days PI for juvenile and adult stages significantly decreased the worm burden, tissue egg load, number of immature egg stages and number of mature eggs with the complete death of eggs. These results are in agreement with the reports of El-Lakkany *et al.*[32] and Abdel-Fattah and Ahmed[33]. In consonance with the report of Aly *et al.*[34], the loss of life of worms following PZQ remedy might also be attributed to metabolic disorders, mechanical destruction and muscular contraction of the dealt with worms. Moreover, percentage reduction in the egg be counted in the contaminated groups handled with PZQ used to be located to be higher in the intestinal tissue than in hepatic tissue. This variant used to be attributed to excretion of some ova from the gut prior to digestion and to hepatic shift of worms aftertreatment[34-36]. Determination of enzyme levels, such as; serum AST and ALT is generally used for the duration of the assessment of liver damage by using schistosomal infection[37,38]. Necrosis or membrane damage released the enzymes into circulation. Therefore, they can be measured in

the serum. In agreement with the reviews of Kadry *et al.*[39], the increment of such enzymes in serum may additionally be due to the destruction of hepatocytes with the aid of the action of toxins of the parasite eggs leading to their release into the circulation. In addition, Naik *et al.*[40] pronounced that hepatocyte membrane damage seems to be the top offender for the marked amplify in the serum marker enzymes; AST, ALT and AFP following schistosomal infection. In conjunction with the report of El-Lakkany *et al.*[32] and Abdel-Fattah and Ahmed[33], facts from the present find out about confirmed that cure with SLNP at the two intervals for juvenile and grown up ranges substantially lowered the stages of serum AST, ALT, AFP and GGT things to do in infected-treated group indicated protection of functional integrity of hepatic telephone membrane. In accord with the studies of El-Lakkany *et al.*[32] and Abdel-Fattah and Ahmed[33], the present study showed that *S. mansoni* infection in mice induced a significant decrease in the serum total protein. In consonance with the report of El-Lakkany *et al.*[32] and Abdel-Fattah and Ahmed[33], information from the current learn about confirmed that remedy with PZQ at the two tested doses for juvenile and person ranges considerably improved the level of complete protein activities in infected-treated group. The anti-schistosomal drug, PZQ motives worm tegument harm that consequently limits or enhances appreciably immune response of sufferers and generates a reversion of the level of fibrosis[14]. Thereby; as evidenced by several studies the significant reduction in oxidative stress initiates a positive impact on the preservation of the liver integrity and function. The major cause of metabolic dysfunction during pathogenesis is the site specific oxidative damage of some of the susceptible amino acids of protein[41]. The AFP of the studied groups showed no statistical significance ($p>0.05$) between the infected untreated control group and PZQ/SLN treated one. This agreed with the result of a previous study[42]. While other studies reported that reduction of the AFP level of infected rats with *S. mansoni* after treatment[37]. Oxidative stress is one of the most common issues in sufferers with continual liver diseases as schistosomiasis[43]. It was once in the past mentioned that throughout schistosome infestation, the parasite tends to switch from Krebs cycle to lactate production in the host which consequences in a surplus provide of O_2 which topics the contaminated host to a country of oxidative stress or extended free radical formation[44]. Moreover, the parasite is uncovered to Reactive Oxygen Species (ROS) generated by means of the host effector cells as macrophages, eosinophils, neutrophils and platelets[45]. The ROS leads to the release of toxic oxygen radicals mainly superoxide anion and H_2O_2 during the respiratory burst. These two radicals may additionally have interaction to produce hydroxyl radical, which is even greater reactive. Schistosomiasis is associated with the liberation of free radicals and disturbance in the cellular antioxidant system. As the infection becomes established, the parasite comes under oxidative stress generated by the host immune system which is counteracted by the parasite antioxidant defense mechanism[46]. The generation of oxygen-derived free radicals may be an initial, non-specific defence reaction of the host toward parasitic infection. In the present study, elevation in the level of the end product NO in both liver and kidney of control group was observed in schistosome infected rats. The significant reduction in oxidative stress initiated a positive impact on the preservation of the liver integrity and function[47]. In the current study, NO content in both kidney and liver of the infected-treated (SnL late+PZQ) group was significantly decreased than infected ($p<0.001$ and $p<0.001$, respectively). Treatment with PZQ/SLN at the two tested doses in the present study significantly decreases the NO level, suggested that the mechanism of PZQ/SLN hepato protection may be due to its antioxidant effect. Hence, PZQ/SLN treatment reduces NO which acts as one of the inflammatory mediators that play a crucial role in schistosomal liver fibrosis and its complications. Hassan *et al.*[47] reported that oxidative stress markers in liver, kidney and spleen showed improvement in infected treated rats in comparison with infected rats. There is increase in NO in kidney and liver of infected untreated group in comparison with controls. It is known that during inflammation and oxidative stress, nitrite/nitrate is couples with O_2 to produce peroxynitrite (ONOO) a very cytotoxic metabolite[48-50]. Schistosomiasis is a primary cause of pulmonary arterial hypertension global and may be paradigmatic of inflammation riding vascular disease. Also, inflammation is the key driver in the pathogenesis of many forms of pulmonary arterial hypertension. However, precise mechanisms by which inflammation results in vascular disease are unknown[51]. Previous studies had reported that transforming growth factor-β signaling is mandatory for multiple forms of experimental pulmonary hypertension because of chronic hypoxia and *Schistosoma* exposure[52]. They found evidence of type-2 inflammation (IL-4 and IL-13) in rats[53] with experimentally induced pulmonary hypertension via *Schistosoma* exposure and deficiency of both IL-4 and IL-13 or treatment with a signal transducer and activator of transcription factor 6 inhibitor, prevented the transforming

growth factor-β induced pulmonary hypertension. Also, chronic schistosomal infection, like that of other helminths, induces a strong type-2 inflammation, thought to be largely mediated by a CD4+T helper 2 (Th2) response, while overturning the type-1 response. This Th2 response, activated by egg-derived antigens, includes cytokines IL-4, IL-5, IL-10 and IL-13. The present study showed a significant decrease in the level of IL-4 between infected control and all other groups either control or treated groups. While there was significant increase in the level of IL-10 between infected group and PZQ treating groups (466 ng mL^{-1} for infected vs. 526,709 ng mL^{-1} for early and late SLN-PZQ and 620 ng mL^{-1} for PZQ only late treated group with $p<0.001$). Increasing the level of IL-10 in infected rats in this study is in agreement with Hesse *et al.*[54], who found that IL-10 was elevated following infection by *S. mansoni*. The anti-inflammatory cytokine IL-10 is pivotal for the generation of hostprotective homeostatic conditions in schistosomiasis[55]. Skin resident tissue macrophages, which encounter *S. mansoni* excretory/secretory products during infection are the first monocytes to produce IL-10 *in vivo* early post infection with *S. mansoni* cercariae[56]. Moreover, IL-10 is essential for maintaining a non-lethal chronic infection and reduces hepatocyte damage induced by the parasite's eggs[57]. Increasing of level of IL-10 in infected rats treated with PZQ in comparison with infected group in this study was in agreement with Wilson *et al.*[58], who found an increase in IL-10 in PZQ-treated humans. This disagrees with Brown *et al.*[59], who reported decline in IL-10 level after treatment with PZQ. These changes in cytokines explain the decrease in hepatic granuloma volume and reflect the anti-inflammatory effects of PZQ/SLN. This agrees with Aly *et al.*[34], who claimed that the increase in IL-10 with PZQ treatment may reduce the granuloma size.

CONCLUSION

Results of the present study showed that the encapsulation of PZQ in SLN improved the safety profile of the drug. Administration of SLN-PZQ at both early stage and late stage of infection was effectively against parasitological and biochemical parameters. The concomitant use of PZQ with SLN showed enhanced therapeutic efficacy compared with that of each one alone. This was evidenced by the nearly complete eradication of immature worms, mature worms and eggs, healing of hepatic granulomatous lesions and normalization of liver serum enzymes levels. The level of oxidative stress marker nitric oxide NO was significantly decreased in treated groups in liver and kidney homogenate.

SIGNIFICANCE STATEMENT

This study discover the nanoformulation of SLN as drug delivery system that can be beneficial after combined with specific drugs for treatment of different infectious diseases.

This study will help the researcher to uncover the critical areas of nanoformulation and use of SLN that many researchers were not able to explore. Thus, a new theory on delivering drugs using SLN nanoformulation may be arrived at.

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