



Evaluation of Poly lactide-co-glycolide Acid [PLGA] Nanoparticles Loaded with Praziquantel in treatment of experimental murine Schistosomiasis mansoni.

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ABSTRACT

Background: Schistosomiasis is a prevailing disease, reported globally in many countries. The classical treatment, although effective, had many disadvantages. Thus, there is a recent interest in nanoparticles [NPs] as a vehicle to increase drug effectiveness and reduce side effects.

Aim of the work: The current study was designed to evaluate the in vivo anti-schistosomal effects of praziquantel-loaded PLGA NPs compared to praziquantel [PZQ] in treatment of experimental murine Schistosomiasis mansoni as regards of worm burden, oogram pattern, tissue egg load, liver enzymes status [AST and ALT], cytokines changes [Interleukin 4(IL-4), Interleukin 10 (IL-10) and Interferon-gamma (IFN-γ)], Interleukin 4 [IL-4] and Interleukin 10 IFN-γ], and histopathological studies [granulomas number and diameter].

Materials and Methods: Eighty mice were classified into eight groups [10 mice each]: normal control, *S.mansoni* infected untreated mice [infected control], infected mice treated with unloaded PLGA NPs on the 14th day post infection [PI], infected mice treated with unloaded PLGA NPs on the 42nd day PI, infected mice treated with PZQ on the 14th day PI, infected mice treated with PZQ on the 42nd day PI, infected mice treated with PZQ-loaded PLGA NPs on the 14th day PI and infected mice treated with PZQ-loaded PLGA NPs on the 42nd day PI. All mice were sacrificed ten weeks PI.

Results: Treated group with PZQ alone on the 42nd day PI showed a significant reduction in total worm burden, reduction in tissue egg count, increase in serum IL-10 level, decrease in serum AST, ALT, IL-4 and IFN-γ levels and reduction in mean hepatic granulomas diameter and number when compared to infected control group. These results were improved when PZQ loaded on PLGA NPs was given on the 42nd day PI showed by no detection of immature ova, a highly significant reduction in the total worm burden in comparison to infected control group. The reduction in mean hepatic granulomas diameter and number and tissue egg count in late PZQ-loaded PLGA NPs group was higher than late PZQ group. While the treated groups with PZQ alone, or loaded on PLGA NPs on the 14th day PI and group treated with unloaded PLGA NPs on the 14th day PI showed non-significant difference regarding total worm burden, tissue egg count, mean hepatic granulomas diameter and number and serum levels of ALT, AST, IL-4, and IL-10 compared to infected control group.

Conclusion: PLGA NPs improved the anti-schistosomal effects of PZQ regarding all assessed parameters, especially a highly significant reduction in mean hepatic granulomas diameter and number by its actions through upregulation of pro-inflammatory cytokines.

Keywords: Poly [lactide-co-glycolide]; Nanoparticles; *Schistosoma mansoni*; Loading; Efficacy of drug.

INTRODUCTION

Human schistosomiasis, one of the most prevalent neglected tropical diseases, is a major public health problem affecting more than 200 million people in 78 countries and ~800 million people are at risk of infection (**Grimes et al., 2015**). It is a helminthic disease caused by intravascular trematode of the *Schistosoma* genus, with three main species adapted to human infection: *Schistosoma mansoni*, *S. haematobium*, and *S. japonicum*. Poor sanitary conditions and low economic development are involved in the disease dissemination (**Rollinson et al., 2013**). Schistosomiasis was recognized by WHO, as the 2nd most important disease, surpassed only by malaria. Thus, schistosomiasis control remains a challenge in endemic regions (**Inobaya et al., 2014**). Egypt has been plagued by Schistosomiasis and it was traditionally the most important public health concern. Treatment of schistosomiasis serves three purposes: reversing acute disease, prevention of complications, and neuroschistosomiasis (**Bahgat, 2014**). The treatment of choice is praziquantel. This drug has the advantage of low cost and wide therapeutic spectrum by operating in adult forms of all species of Schistosomes. On the contrary, it does not exert any effective action on immature worms, due to the fast metabolism of praziquantel after oral intake. The drug is converted to an inactive or less potent metabolite (**Liu et al., 2017**). Also, the presence of younger forms in the systemic circulation, leads to the reduction of exposure to praziquantel. So, it is necessary that, high oral doses are needed to overcome first-pass metabolism and achieve sufficient drug concentrations in the larval tissue. Furthermore, the low solubility and bioavailability of praziquantel lead to limitation of its use. Thus, many trials had described different technological substitutes to improve the oral absorption of this drug and used new compounds in the prevention and treatment of this disease

(**De Souza et al., 2014**).

Due to its poor solubility in water, low oral bioavailability, and risk of development of resistance to praziquantel, it would be essential to develop a novel product that can overcome these shortcomings by making praziquantel an excellent candidate for encapsulation in solid lipid nanoparticles (**Pérez del Villar., 2012**). PLGA NPS have the advantages of different colloidal carriers and also avoid some of their disadvantages (**Sun et al., 2019**).

This study aims to evaluate the in vivo anti-schistosomal effects of PZQ-loaded PLGA NPs compared to PZQ alone in treatment of experimental murine schistosomiasis mansoni regarding the following parameters: parasitological parameters [worm burden, oogram pattern and tissue egg load], biochemical parameters [AST, and ALT], immunological parameters [IL-4, IL-10 and IFN- γ], and histopathological parameters [granulomas number and diameter].

MATERIAL AND METHODS:

The study was held at the Biological Supply Program Unit for *Schistosoma mansoni* in Theodor Bilharz Research Institute [SBSP/TBRI] Giza, Egypt, in firm agreement with the TBRI ethical guidelines for use of animals in research. This study was conducted on eighty laboratory Swiss albino female mice 6-8 weeks old. They were clean from parasitic infection, weighed 20 ± 2 g at the beginning of the experiment. Animals were housed in conditioned rooms with a stable temperature at 21°C, with free access to sterile water *ad libitum*, and fed a balanced diet with 14% protein.

S. mansoni cercariae

S. mansoni cercariae were obtained from infected *Biomphalaria alexandrina* snails reared and maintained at SBSP/TBRI. Mice were infected subcutaneously with freshly emerged cercariae in dose of 80 ± 10 *S. mansoni* cercariae/mouse according to the (**Peters and**

Warren, 1969).

Preparation of PLGA Nanoparticles:

PLGA; Poly (D,L-lactide-co-glycolide) [50:50, M_w . 17000] [Sun Pharma, Vadodara]. Dichloromethane [DCM], acetone, polyvinyl alcohol [PVA], Triton X-100, dialysis membrane [MWCO 6,000 -7,000 Da] were purchased from Himedia, Mumbai, India. All solvents and reagents used were of analytical grade unless stated otherwise. Double distilled water [DDW] was used in the study.

Method of preparation:

Empty NPs were prepared by the double emulsion-solvent evaporation technique. PLGA was dissolved in a mixture of dichloromethane [DCM] and acetone then polyvinyl alcohol [PVA] solution was added. Further mixture was emulsified by sonication for 60 seconds in an ice bath and water-in-oil [w/o] emulsion was formed. Again PVA solution was added and sonicated for 60 seconds in an ice bath and water-in-oil-in-water [w/o/w] emulsion was formed. This resulting water-in-oil-in-water [w/o/w] emulsion was again diluted with 20 ml of PVA solution and slow speed stirring was continued for 5 hours to facilitate evaporation of the solvent. The NPs so formed were collected, washed, and freeze-dried (Chitkara and Kumar, 2013).

Praziquantel:

Biltricide, [Alexandria Co. for Pharmaceuticals and Chemical Industries] is available as tablets and each tablet contains 600 mg of PZQ (active ingredient). PZQ was given to mice orally in the form of an aqueous suspension in 2% Cremophore-EI solution (Sigma-Aldrich Chemical Co, St. Louis, MO, USA) freshly prepared immediately prior to administration to the mice by gastric gavage in a dose of 500 mg/kg/day (Gonnert and Andrews, 1977).

Preparation of PZQ-loaded nanoparticles:

The drug was loaded at the initial step of NPs

preparation. The drug was dissolved in 1.5% PVA solution, and then was added to the dissolved PLGA solution by syringe and further steps were followed as in the case of unloaded NPs.

Study design:

Eighty female Swiss albino mice were classified into eight groups [10 mice/group] as follows:

- Group 1: Normal control received a normal diet.
- Group 2: Infected mice with *S.mansoni* and received a normal diet [infected control].
- Group 3: Infected mice with *S.mansoni* then received a single dose of unloaded PLGA NPs [50 µg/kg] on the 14th day PI. [Early PLGA NPs -treated group].
- Group 4: Infected mice with *S.mansoni* then received a single dose of unloaded PLGA NPs on the 42nd day PI. [Late PLGA NPs -treated group].
- Group 5: Infected mice with *S.mansoni* then received a single dose of PZQ on the 14th day PI. [Early PZQ-treated group].
- Group 6: Infected mice with *S.mansoni* then received a single dose of PZQ on the 42nd day PI. [Late PZQ-treated group].
- Group 7: Infected mice with *S.mansoni* then received a single dose of PLGA NPs loaded with PZQ on the 14th day PI. [Early PZQ-loaded PLGA NPs treated group].
- Group 8: Infected mice with *S.mansoni* then received a single dose of PZQ-loaded PLGA NPs on the 42nd day PI. [Late PZQ-loaded PLGA NPs treated group].

All mice in different groups were deprived of food the night before treatment and they allowed to eat 1 h after treatment.

Scarification:

Mice of all experimental groups were

sacrificed ten weeks PI by decapitation without anesthesia, and were subjected to the following investigations.

Worm burden:

Adult worms of *S.mansoni* were reaped from each sacrificed mouse by the perfusion of hepatic and portomesenteric vessels, and then the collected worms were counted (**Duvall and De Witt, 1967**). The reduction in numbers of worms post-treatment was calculated (**Tendler et al., 1986**).

Tissue egg load in liver and intestine:

The eggs number per one gram liver or intestinal tissue was detected by weighing a part of intestine or liver, then, it is digested and incubated in 5% potassium hydroxide overnight (**Cheever, 1968**). The eggs number per gram of intestinal or hepatic tissue were calculated by multiplying the average eggs number in each 1 ml sample by the total volume of potassium hydroxide and then dividing that value by the sample weight.

Oogram Patterns:

The whole small intestine of each sacrificed mouse was removed, transferred to a petri dish and opened lengthwise into 3 parts (each 1 cm in length), and then squeezed between a standard glass slide and coverslip to identify and count various developmental stages of *S. mansoni* eggs [immature, mature and dead eggs] in the three samples from each mouse in each part, and the mean number of eggs of each developmental stage/animal was determined (**Pellegrino et al., 1962**).

Histopathological study:

Livers were removed from mice, washed by normal saline, fixed in 10% formalin and embedded in paraffin blocks. To measure granuloma, a 5 μ m thickness sections were cut by microtome, with a distance of 250 μ m apart to

prevent re-measurement. Then, hematoxylin and eosin stains were used to stain sections. The counting of hepatic egg granulomas was achieved in five microscopic fields in serial tissue sections. The diameter of liver granulomas was measured by the ocular micrometer. The mean granuloma diameter was estimated by dividing the sum of perpendicular diameters (vertical and transverse diameters)/2. The granuloma size is expressed as the mean area measured in $\mu\text{m}\pm\text{SD}$. Only lobular granulomas (non-confluent) containing ova in their centers were measured (**Von Lichtenberg, 1962**).

Serum preparation:

For serological investigations, blood samples from each mouse were collected through a small puncture of the caudal vein using a sterile needle. Samples were transferred to a 1.5 ml tube, clotted, and then centrifuged for serum separation. Serum samples were kept at -20°C until used.

Assessment of serum liver enzymes:

Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were analyzed using a commercial kit [Boehringer reagent kit, Mannheim, Germany] according to the manufacturer's instructions (**Reitman and Frankel, 1957**).

Cytokine assays:

Serum Interleukin 4 (IL-4), Interleukin 10 (IL-10) and Interferon-gamma (IFN- γ) concentrations were determined by the enzyme linked immunosorbent assays according to the manufacturer's instructions [BioSource International, Inc., Camarillo, California, USA]. for, IL- 4, IL-10 and IFN- γ .

Statistical analysis:

Collected data were arranged, coded, tabulated and introduced to a PC using the Statistical Package for Social Science [SPSS] for windows version 18.0. Data were represented as

the mean \pm standard deviation (SD). The analysis of variance [ANOVA] procedure was used to clarify statistically significant differences between the different studied groups. P value was considered statistically significant when $P < 0.05$.

RESULTS

Worm burden: late PZQ-loaded PLGA NPs infected treated group demonstrated a highly significant reduction in mean total worm burden followed by late PZQ treated group, while other infected treated groups showed non-significant differences in comparison to infected control group **Table (1)**.

Oogram patterns: late PZQ-loaded PLGA NPs treated group showed the highest elevation of the percentage of dead ova (95.31%) with complete disappearance of immature ova followed by late PZQ treated group in comparison to infected control group **Table(2)**.

Hepatic tissue egg load: There was a reduction in ova count in hepatic tissue of the infected treated groups; late PZQ and late PZQ-loaded PLGA NPs when compared with infected control group. The reduction in hepatic tissue egg load in late PZQ and late PZQ-loaded PLGA NPs infected treated mice groups was 94.2% and 97.1% respectively, with a highly significant difference ($P < 0.001$), while other infected treated groups showed non-significant reduction in comparison to infected control group **Table (2)**.

Intestinal tissue egg load: There was a reduction in ova count in intestinal tissue in late PZQ and late PZQ-loaded PLGA NPs in comparison with infected control group. The reduction in intestinal tissue egg count in late PZQ and late PZQ-loaded PLGA NPs infected treated mice groups was 95.8% and 98.1% respectively, with a highly significant difference ($P < 0.001$), while other infected treated groups showed non-significant reduction in comparison to infected control group **Table(2)**.

Biochemical Parameters:

Liver enzymes: The measured levels of serum AST and ALT were found to be significantly increased in infected controls in comparison to normal controls. At the same time, treated groups with PZQ alone or loaded on PLGA NPs at the 42nd day PI showed highly significant decrease in levels of AST and ALT in comparison to infected control group. The decrease in AST and ALT levels in late PZQ-loaded PLGA NPs infected treated group was higher than late PZQ treated group. While other infected treated groups showed non-significant decrease in comparison to infected control group **Table(3)**.

Serum cytokines assay: The measured levels of cytokines (IL-4, IL-10 and IFN- γ) were found to be significantly increased in infected mice in comparison to normal controls. While the treated group with PZQ alone on the 42nd day PI and treated group with PZQ-loaded PLGA NPs on the 42nd day PI showed highly significant decrease in IL-4 level and highly significant increase in IL-10 level in comparison to infected controls. On the other hand, there was a significant decrease in IFN- γ levels was detected in all treated groups in comparison to infected control group **Table(4)**.

Stained liver sections showed reduction in mean diameter of hepatic granulomas in late PZQ and late PZQ-loaded PLGA NPs treated groups was (73.5% and 97.6%) respectively, with highly significant difference ($P < 0.001$) in comparison to infected control group. The reduction in mean granuloma number in late PZQ and late PZQ-loaded PLGA NPs treated groups was (76.1% and 90.3%) respectively, with highly significant difference [$P < 0.001$] in comparison to infected control group. While other infected treated groups showed non-significant reduction in comparison to infected control group **Table (5)**.

Table(1): Effect of different treatment regimens on worm burden in *Schistosoma mansoni* infected mice in comparison to infected control group.

Groups	Parameters	Total worm burden Mean± SD (R %)	P value
	Infected control	17.1±1.97	
	Early unloaded PLGA NPs treated	16.5±1.7 (R 3.51%)	0.48(NS)
	Late unloaded PLGA NPs treated	15.9±1.86 (R 7.01%)	0.18(NS)
	Early PZQ treated	16.2±1.22 (R 5.26%)	0.24(NS)
	Late PZQ treated	4.8±1.74 (R 71.92%)	<0.01*
	Early PZQ-loaded PLGA NPs treated	15.8±1.46 (R 7.60%)	0.11(NS)
	Late PZQ-loaded PLGA NPs treated	1.3±1.26 (R 92.39%)	<0.001**

(NS): Non-significant, (SD): Standard deviation, (R%): percent of reduction, *significant, **highly significant

Table (2): Effect of different treatment regimens on oogram patterns and ova count in tissues in *Schistosoma mansoni* infected mice in comparison to infected control group.

Groups	Parameters			Tissue egg load (R%)			
	Oogram patterns (% of ova developmental stages)			Liver		Intestine	
	Immature	Mature	Dead	Mean± SD	P value	Mean± SD	P value
Infected control	50.6	42.33	7.07	8995.2±1201.75	-	12485.8±3741	-
Early unloaded PLGA NPs treated	45.31	45.43	9.26	8959.2±1338.1 (R 0.4%)	0.95	12448.3±612.19 (R 0.3%)	0.98
Late unloaded PLGA NPs treated	44.56	43.23 %	12.21	8860.3±1201.3 (R 1.5%)	0.80	12432.3±1236.28 (R 0.4%)	0.97
Early PZQ treated	41.31	46.11	12.58	8735.9±901.1 (R 2.9%)	0.59	11789.6±1189.1 (R 5.6%)	0.58
Late PZQ treated	2.38	4.53	93.09	521.7±29.54 (R 94.2%)	<0.001**	524.4±705.52 (R 95.8%)	<0.001**
Early PZQ-loaded PLGA NPs treated	39.31	43.91	16.78	8514.3±1313.3 (R 5.4%)	0.4	11624.3±703.05 (R 6.9%)	0.48
Late PZQ-loaded PLGA NPs treated	0%	4.69	95.31	260.3±12.89 (R 97.1%)	<0.001**	237.3±12.36 (R 98.1%)	<0.001**

Tables (3): Effect of different treatment regimens on liver enzymes activities of *S. mansoni* infected mice in comparison to infected control group.

Groups	Parameters	ALT Mean± SD	P value	AST Mean± SD	P value
	Normal control	46.9±4.13	-	42.9±2.86	-
	Infected control	71.1±5.38	<0.001**	69.28±3.86	<0.001**
	Early unloaded PLGA NPs treated	68.95±8.93	0.52	67.7±5.75	0.48
	Late unloaded PLGA NPs treated	67.3±7.46	0.21	66.9±6.22	0.32
	Early PZQ treated	65.1±8.32	0.07	64.88±8.99	0.17
	Late PZQ treated	45.1±5.86	<0.001**	41.29±7.81	<0.001**
	Early PZQ-loaded PLGA NPs treated	66.0±8.32	0.14	60.8±8.9	0.21
	Late PZQ-loaded PLGA NPs treated	44.8±6.55	<0.001**	38.3±6.52	<0.001**

Table (4): Effect of different treatment regimens on level of serum IL-4, IL-10 and IFN- γ of *S. mansoni* infected mice in comparison to infected control group.

Parameters Animal groups	IL-4 Pg/ml Mean \pm SD	P value	IL-10 Pg/ml Mean \pm SD	P value	IFN- γ Pg/ml Mean \pm SD	P value
Normal control	14.39 \pm 0.68		213.8 \pm 30.89	-	229.8 \pm 29.3	
Infected control	70.2 \pm 11.2	<0.001**	508.3 \pm 29.16	<0.001**	657.8 \pm 29.21	<0.001**
Early unloaded PLGA NPs treated	61.19 \pm 13.12	0.12	511.5 \pm 45.56	0.86	503.13 \pm 21.91	<0.001**
Late unloaded PLGA NPs treated	59.9 \pm 12.51	0.07	513.8 \pm 46.53	0.76	430.26 \pm 45.9	<0.001**
Early PZQ treated	60.8 \pm 10.21	0.7	516.6 \pm 50.35	0.58	480.1 \pm 32.1	<0.001**
Late PZQ treated	46.4 \pm 8.62	<0.001**	578.4 \pm 21.91	<0.001**	616.51 \pm 31.3	0.007**
Early PZQ-loaded PLGA NPs treated	61.1 \pm 13. 1	0.12	517.3 \pm 41.51	<0.58	520.32 \pm 41.21	<0.001**
Late PZQ-loaded PLGA NPs treated	21.63 \pm 5.9	<0.001**	585.9 \pm 36.32	<0.001**	622.65 \pm 24.32	0.009**

Table (5): Effect of different treatment regimens on mean hepatic granulomas diameter and number of of *S. mansoni* infected mice in comparison to infected control group.

Parameters Animal groups	Granuloma diameter		Granuloma number	
	Mean \pm SD (R %)	P value	Mean \pm SD (R%)	P value
Infected control	331 \pm 78.5	-	11.3 \pm 2.5	-
Early unloaded PLGA NPs treated	316 \pm 87.2 (R 4.5%)	0.69	10.7 \pm 2.6 (R 5.3%)	0.61
Late unloaded PLGA NPs treated	301.2 \pm 74.9 (R 9%)	0.4	9.8 \pm 2.3 (R 13.3%)	0.18
Early PZQ treated	287.5 \pm 75.6 (R 13.1%)	0.22	10.7 \pm 1.9 (R 5.3%)	0.55
Late PZQ treated	87.7 \pm 5.1 (R 73.5%)	<0.001	2.7 \pm 0.9 (R 76.1%)	<0.001
Early PZQ-loaded PLGA NPs treated	280.8 \pm 63.3 (R 15.2%)	0.13	9.5 \pm 2.1 (R 15.9%)	0.1
Late PZQ-loaded PLGA NPs treated	7.8 \pm 0.9 (R 97.6%)	<0.001	1.1 \pm 0.6 (R 90.3%)	<0.001

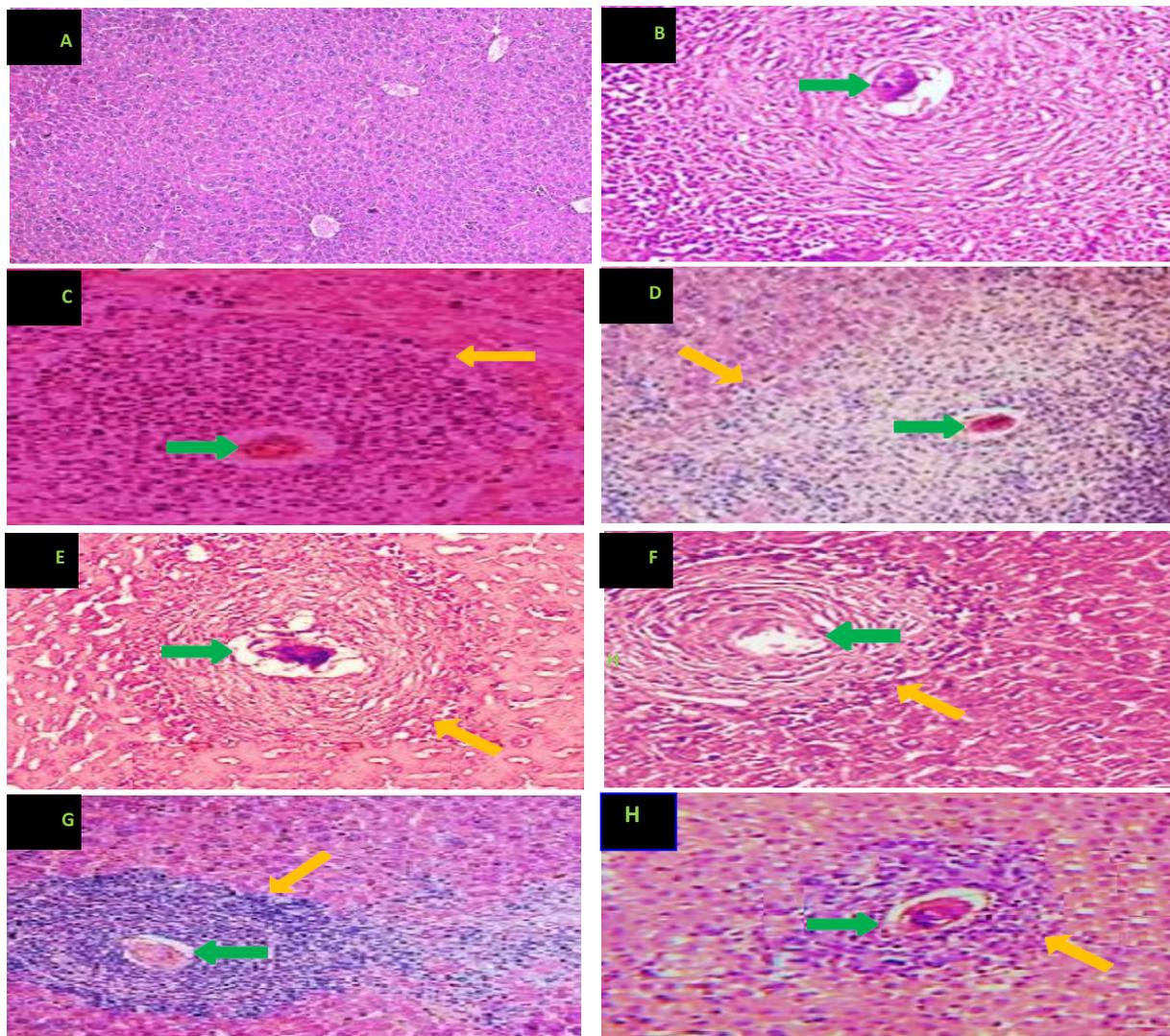


Figure (1): Histopathological study of H&E-stained liver sections of different groups of mice (X 100).

[A] Normal control shows the normal architecture of hepatocytes which appears radiating from the central vein. The hepatocytes have an open face nucleus and acidophilic cytoplasm. Neither inflammatory cells nor granulomas are seen in the liver. [B] Infected control shows large granuloma of fibrocellular type. The egg is viable showing central miracidium. [C] Early unloaded PLGA NPs shows large granuloma of cellular types with dense epithelioid cells component and few lymphocytes with a viable egg. [D] Late unloaded PLGA NPs shows large granuloma of cellular types with dense epithelioid cells component and few lymphocytes. The egg is viable. [E] Early PZQ shows regressed granuloma markedly fibrosed with few epithelioid cells and few lymphocytes. The egg is partially degenerated. [F] Late PZQ shows resolved granuloma with marked fibrosis and few epithelioid cells and few lymphocytes. The egg is partially degenerated with moderate decrease in granuloma diameter. [G] Early PZQ-loaded PLGA NPs shows large granuloma of cellular types with dense lymphocyte component and few epithelioid cells. The egg is viable. [H] Late PZQ-loaded PLGA NPs shows small granuloma of fibrocellular types with few epithelioid cells and few lymphocytes. The egg is markedly degenerated with a marked decrease in granuloma diameter.

DISCUSSION

Schistosomiasis, is a neglected tropical parasitic disease, it is endemic in 78 countries, that afflicts more than 240 million people (**Santos et al., 2014**). A vaccine for schistosomiasis is not yet present and chemotherapy depends on praziquantel which is very effective and safe. However, it is not devoid of disadvantages (e.g., stage-dependent susceptibility and poor efficacy against immature stages). Thus, there is a crucial need to search for new anti-schistosomal drugs. Recently, nanoparticles have gained more attention in the field of anti-parasitic medications (**Yah and Sinate, 2015**).

The anti-Schistosomal treatment was advocated as it delivers many supplementary goals, decrease egg-stimulated pathology, minimal changes of parenchyma with the eradication of worms (**EI-Lakkany et al., 2011**).

The preceded researches concentrate on the epidemiological and physiological aspects of schistosomiasis. But, neglect the metabolic alterations evolved in the host as a sequelae of infection or medical PZQ) treatment. Thus, the evaluation of the treatment efficacy of nanoparticles loaded with PZQ in infected rats is important for the assessment of the infection extent and effectiveness of treatment. Measurement of different enzyme levels [e.g., serum AST and ALT] is largely used to assess liver cell damage caused by schistosomal infection (**Aly and Mantawy, 2013**).

In line with **Mahmoud and Elbessouny (2013)** study, the increase of serum levels of liver enzymes may be due to the damage of hepatocytes, due to parasite egg toxins effects. Furthermore, (**Naik et al., 2011**) reported that, damage of hepatocyte membrane looks to be the

prime culprit for the significant increase in the serum enzymes, AST, ALT, and AFP after schistosomal infection.

The present work detected that the administration of PZQ-loaded PLGA NPs on the 42nd day PI significantly reduced the total worm burden, tissue egg count and granulomas diameter and number. These results are in harmony with the study of (**Rabia et al., 2010**) who detected PZQ fatal effect on the worms, which may be due to metabolic effects, mechanical damage and muscular contraction of the worms subjected to treatment. (**EI- Moslemany et al., 2016**) used a miltefosine encapsulation technique in LNCs in *S. mansoni* - infected mice. Their results revealed that, a single oral dose of encapsulated miltefosine led to decrease parasite load and reduce hepatic granulomas in infected mice. Similarly, (**EI-Beshbishi et al., 2013**) reported that hepatic tissues of untreated-infected mice 6 weeks PI showed granulomata encircling recently deposited intact or partially degenerated ova with a mean number of 14.50 ± 2.07 and a diameter of $288.83 \pm 46.44 \mu\text{m}$. However, in the PZQ group, the parenchymal changes were similar to those in the infected untreated controls, with the least reduction in the granuloma number and diameter of 36.55 and 22.34%, respectively.

In our current study, the histopathological examination of the liver sections in late PZQ treated group revealed a reduction in the diameter of the liver granulomas in comparison with the infected control group in accordance with (**EI-Lakkany and Nosseir, 2007**).

PZQ exert significant regulatory actions on responses of cell immune which increases CD8 cells and decreases CD4 T cells, with the subsequent result of reduction in size of hepatic granuloma in schistosomiasis as described by (**EI-**

Ahwany et al., 2006).

Several studies revealed that PZQ is an effective medication to control infection with *S. mansoni*, stimulating a decrease in egg excretion and reversion of fibrosis in mice and humans (Martins-Leite et al., 2008). Probably, the increase in T cell reactivity after treatment can be interpreted by the exposure of liberated antigens to the host immune system after the destruction of worms by drugs.

Moreover, the percentage of decrease in the egg count in the infected-treated groups was found to be high in the intestinal than in hepatic tissue. This difference was belonging to the excretion of some ova from the intestine prior to digestion and to the shift of worms to the liver after treatment (El-Sayed et al., 2016).

In the current work, IL-4, and IL-10 displayed a highly significant increase in the infected control group in comparison with normal control group indicating that, immune reactions to schistosome in chronically infected patients are typically shifted toward Th2 cytokines. Also, IL-4 significantly decreased and IL-10 significantly increased in late PZQ and late PZQ-loaded PLGA NPs groups in comparison to infected control group. It has been suggested that IL-4 upregulates chemokine, fibroblast, collagen and matrix protein, which signify that this cytokine is a crucial cytokine for granuloma formation (Liu et al., 2002). This is agreed with that of (Franchimont et al., 1999) who demonstrated that administration of exogenous IL-10 resulted in a reduction of granuloma size. In addition, an opposite effect was seen in IL-10-deficient mice (Sanin et al., 2015). Nevertheless, type 2-related cytokines such as IL-4 and IL-10 impede classical activation of macrophage and has been involved in the formation of granulomas and fibrogenesis around eggs deposited in the tissues (Colley

and Secor, 2014).

In the current study, there was significant difference in the IFN- γ in the infected treated group than in the infected control group. This result was correlated with (Boros, 1994) who stated that IFN- γ was found to be produced very early at the inception of the liver granulomatous response, by the time granulomas reached the maximal size, and at the same time, its production reduced. In addition, (Silva et al., 2004) supported and proposed that there was a significant general downregulation of responses of Th1 cells in mice infected with *Schistosoma*. (Caldas et al., 2008) reached to the conclusion that, there was a mixed Th1 and Th2 cytokine expression during response in the acute phase, with a predominance of Th1 in the early infection. Also the excess production of IFN- γ , in the acute phase in comparison with chronic phase schistosomiasis, partially explained the lack of immune response modulation in the acute infections. All these responses may be due to an intense negative action of parasite eggs on schistosome antigen driven IFN- γ production. Also, (Boros and Lukacs, 1992) reported that IFN- γ decreased the granulomas cellularity and reduced the size of granuloma in experimental schistosomiasis. These data are in line with (De Jesus et al., 2004) who proposed that IFN- γ is the principle Th1 cytokine included in the reduction of Th2 cells. It was reported that IFN- γ plays a crucial role in the protective mechanism against periportal fibrosis (Kamdem et al., 2018).

In conclusion, PLGA NPs improved the anti-schistosomal effects of PZQ on adult *Schistosoma mansoni*, especially a highly significant reduction in number and diameter of hepatic granulomas by its action through upregulation of pro-inflammatory cytokines.

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الملخص العربي

تقييم تأثير البرازيكوانتيل المحمل على الجزيئات النانوية PLGA في علاج فئران التجارب المصابة بمرض البلهارسيا المعوية (المنسونية).

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خلفية البحث: مرض البلهارسيا هو أحد الأمراض المنتشرة على مستوى العالم في العديد من البلدان. العلاج التقليدي على الرغم من فعاليته إلا أن له العديد من العيوب. وبما أنه يوجد هناك اهتمام حديث بالجزيئات النانوية كوسيلة لزيادة فعالية الدواء وتقليل آثاره الجانبية.

هدف الدراسة: تقييم تأثير البرازيكوانتيل المحمل على الجزيئات النانوية مقارنة مع البرازيكوانتيل غير المحمل في علاج فئران التجارب المصابة بمرض البلهارسيا المنسوية فيما يتعلق بالعبء الكلي للديدان، ونمط وشكل البويضات وعدد البويضات في الأنسجة، مستوى إنزيمات الكبد ومستوى انترلوكين ٤، انترلوكين ١٠ و انترفيرون جاما ، ودراسة الحالة المرضية لأنسجة الكبد عن طريق تحديد عدد الأورام الحبيبية وقياس قطرها.

المواد وطرق البحث: تم تقسيم ثمانين فأراً إلى ثماني مجموعات [١٠ فئران لكل مجموعة]: المجموعة الضابطة السليمة، المجموعة الضابطة المصابة بمرض البلهارسيا المنسوية ، المجموعة المصابة المعالجة بالجزيئات النانوية PLGA في اليوم الرابع عشر بعد الإصابة، مجموعة الفئران المصابة المعالجة بالجزيئات النانوية PLGA في اليوم ٤٢ بعد الإصابة، مجموعة الفئران المصابة المعالجة بالبرازيكوانتيل في اليوم الرابع عشر بعد الإصابة ، مجموعة الفئران المصابة المعالجة بالبرازيكوانتيل في اليوم ٤٢ بعد الإصابة ، مجموعة الفئران المصابة المعالجة بالجزيئات النانوية PLGA المحملة بالبرازيكوانتيل في اليوم الرابع عشر بعد الإصابة واخيراً مجموعة الفئران المصابة المعالجة بالجزيئات النانوية PLGA المحملة بالبرازيكوانتيل في اليوم ٤٢ بعد الإصابة. تم ذبح جميع الفئران بعد مرور عشرة أسابيع بعد الإصابة.

النتائج: أظهرت المجموعة المعالجة بالبرازيكوانتيل فقط في اليوم ٤٢ من بعد الإصابة انخفاضاً ذا دلالة إحصائية في العبء الكلي للديدان ، وانخفاض عدد البويضات في الأنسجة ، وزيادة مستوى انترلوكين ١٠ في الدم ، وانخفاض في مستويات انزيم AST ، وانزيم ALT ، و انترلوكين ٤ ، و انترفيرون جاما وانخفاض متوسط قطر وعدد الأورام الحبيبية الكبدية بالمقارنة مع المجموعة الضابطة المصابة. ثم تحسنت هذه النتائج عندما تم تحميل البرازيكوانتيل على الجزيئات النانوية PLGA في اليوم الثاني والأربعين بعد الإصابة والذي أظهر اختفاء البويضات غير الناضجة ، وانخفاض كبير في العبء الكلي للديدان مقارنة بالمجموعة الضابطة المصابة. وكان الانخفاض في متوسط قطر الأورام الحبيبية الكبدية وعدد البويضات في الأنسجة في المجموعة المعالجة بالجزيئات النانوية PLGA المحمل بالبرازيكوانتيل في اليوم ٤٢ بعد الإصابة أعلى منها في المجموعة المعالجة بالبرازيكوانتيل في اليوم ٤٢ بعد الإصابة . بينما لم تظهر المجموعات المعالجة بالبرازيكوانتيل وحده ، أو المحملة على الجزيئات النانوية PLGA في اليوم الرابع عشر بعد الإصابة والمجموعة التي عولجت بالجزيئات النانوية PLGA غير المحملة في اليوم الرابع عشر أو ٤٢ بعد الإصابة فروقاً ذات دلالة إحصائية فيما يتعلق بعبء الدودة الكلي ، وعدد البويضات في الأنسجة ، ومتوسط قطر الأورام الحبيبية الكبدية وعددها ومستويات AST و ALT و انترلوكين ٤ وانترلوكين ١٠ مقارنة بالمجموعة الضابطة المصابة.

الاستنتاج: تشير نتائج هذه الدراسة إلى ان الجسيمات النانوية PLGA تحسن عمل البرازيكوانتيل كعلاج فعال ضد مرض البلهارسيا في الفئران فيما يتعلق بجميع المعايير التي تم تقييمها ، لا سيما الإنخفاض الكبير في متوسط قطر الأورام الحبيبية الكبدية وعددها من خلال التنظيم الإيجابي للسيتوكينات المحفزه (المنشطة) للالتهابات.