The anti-schistosomal activity of magnetite and zero-valent iron nanoparticles on *Schistosoma mansoni*: An *in vivo* study

Original Article

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ABSTRACT

Background: Depending mainly on Praziquantel (PZQ) for treatment of schistosomiasis poses a great challenge in terms of effectiveness and resistance. Nanoscale particles formed by metals as iron nanoparticles (INPs) have recently gained approval from the Food and Drug Administration for use as therapeutic agents. Therefore, INPs application as potential therapeutic agents against schistosomiasis may give promising results.
Objective: The present study aimed at assessing the efficacy of INPs; iron oxide or magnetite INPs (MNPs) and zero-valent INPs (ZV-INPs) on *S. mansoni* using parasitological and histopathological parameters.
Material and Methods: In the current study, MNPs and ZV-INPs were prepared by biogenic synthesis and were given to mice orally on the 42nd day post infection (dpi) with *S. mansoni* in a dose of 10 mg/kg for four consecutive doses. The therapeutic efficacy was assessed using parasitological (mortality rate, adult worm load as well as female fecundity) and histopathological parameters (tissue egg count in both liver and intestine) in comparison to untreated and PZQ treated control groups.
Results: Results revealed that ZV-INPs have a significant effect in decreasing both tissue egg count and hepatic granulomata size. While the MNPs have a significant effect against the total and female worms burden, tissue egg counts, female fecundity, and number of liver granuloma.
Conclusion: Herein, it was concluded that both types of INPs used in the study are potentially effective anti-schistosomal agents.

Keywords: iron nanoparticles, magnetite INPs, praziquantel, *S. mansoni*, zero-valent INPs.

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INTRODUCTION

Schistosomiasis is one of the most prevalent diseases worldwide, as its transmission was reported from several countries. The World Health Organization (WHO) estimates showed that at least 236.6 million people required preventive treatment in 2019 to reduce and prevent morbidity[1]. Even though morbidity and mortality due to schistosomiasis declined over the past two decades, effective control of schistosomiasis remains a hard task for populations living in endemic areas of the tropical and subtropical regions of the world[2]. The complexity of *Schistosoma* life cycle stages leads to a complicated list of requirements to obtain a successful control program, that includes chemotherapeutic measures as the main pillar in control of schistosomiasis[3]. For almost 50 years, schistosomiasis treatment depended mainly on a single drug of choice, i.e. PZQ. However, there are reports of emerging resistant strains of schistosomes possibly due to extensive PZQ use in both targeted treatment as well as mass drug administration programs[4]. Therefore, there is an urgent need to discover new effective anti-schistosomal drugs with high efficacy and low resistance.

Metallo-pharmaceuticals, that are considered as a revolution in the field of research, revealed advantages for use especially in the biomedical field[5]. Its application together with nanotechnology, that deals with construction of the material dimension between 1 and 100 nm according to National Science Foundation of USA, enlightened a road to modern biomedicine. Owing to their small size, NPs have improved solubility, absorption, and uptake. Therefore, NP-based medicines can get past cell membranes and reach specific targets more easily than bulk form agents[6]. Silver and gold NPs were the most common elements used as anti-parasitic agents and contributed to many publications in the past decade[7]. Silver NPs are the most studied and considered the first metal NPs approved therapeutically for their anti-inflammatory, anti-cancer and anti-infectious properties. Furthermore other metallic NPs were tested and interpreted for their odds and equalities to silver type[8]. Concerning the side effects of using heavy metal NPs as anti-parasitic agents, literature review articles concluded three important facts. First, NPs cytotoxicity is dependent on the individual physicochemical properties and environmental conditions. Second, NPs can affect cell growth and

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viability in a dose-dependent manner. Third, the most important factor of NPs toxicity is their stability, both *in vivo* and during synthesis and storage[7-9].

Non-oxidized ZV-INPs are widely used in environmental research due to their ability to produce high-energy reactive oxygen species. The latter can overcome and degrade organic pollutants that are non-decomposable from the environment. In the field of medicine, the incorporation of ZV-INPs with silver targeting malignant cells, led to malignant cell apoptosis and autophagy[6]. On the other hand, magnetic NPs, especially iron oxide or MNPs, became of new interest for scientists due to the phenomenon of super-paramagnetism. They obey the Coulomb's law of electrostatic force interaction[10], where they can be directed to active sites *in vivo* under the influence of an external electromagnetic field[11]. Hence, ferrimagnetic iron oxide NPs gain medical interest especially in the field of diagnostics. In the field of therapeutics, ferumoxytol is a newly modified product that is approved for treatment of anemia[12], and is considered as a promising drug in treatment of cancer due to its effect on macrophage polymerization[13].

In the field of infectious diseases, MNPs have shown promising potential for the delivery of certain bactericidal agents to highly restricted microenvironments[14]. This mainly depends on the phenomenon of magnetic fluid hyperthermia, i.e., the application of a fluctuating magnetic field makes magnetic NPs dissipate energy as heat, causing a localized increase in temperature around them[15]. However, several studies proved that the intrinsic antimicrobial properties of MNPs and ZV-INPs are due to the generation of reactive oxygen species that damage microbes' DNA, RNA and proteins[16-18].

In the scope of medical parasitology, super-paramagnetic iron oxide NPs introduced a new non-invasive tracking technique for *E. histolytica* trophozoites *in vivo*[19]. Besides, MNPs showed their effectiveness in separation of *P. falciparum* infected erythrocytes from non-infected ones[20]. Iron oxide in the form of beads showed acceptable sensitivity and feasibility in diagnosis of schistosomiasis through the detection of *Schistosoma* antigens using ELISA[21]. At the therapeutic level, magnetic fluid hyperthermia proved to play a role as an action mechanism of NPs against the protozoan parasite *L. mexicana in vitro*[22].

Finally, INPs were previously tested *in vitro* on adult *S. mansoni* worms and results showed several tegumental derangements revealed by scanning electron microscopy. Besides, the NPs proved to increase the mortality rate of *B. alexandrina* snails[23]. Thus, we designed the present study to interpret the efficacy of both ZV-INPs and MNPs in comparison to PZQ against experimental schistosomiasis *mansoni*, using parasitological and histopathological parameters.

MATERIAL AND METHODS

This case-control experimental study was conducted during the period from January to April 2021. Mice infection and all parasitological and histopathological assessments were performed at the laboratory of Medical Parasitology Department, Faculty of Medicine, Alexandria University, Alexandria, Egypt. Biosynthesis of INPs was performed in the Laboratory of the City of Scientific Research and Technological Applications (SRTA-City), Alexandria.

Study design: Forty mice were experimentally infected with *S. mansoni* cercaria, and, were equally divided into four groups (Table 1). Stool examination was performed to confirm absence of any other parasitic infection before starting the treatment. Iron NPs were prepared by biogenic synthesis due to its safety and low expense when compared to traditional synthesis methods. A pilot study was conducted to determine the least effective dose of MNPs and ZV-INPs capable of decreasing the total worm burden, and 10 mg/kg/d for four consecutive days was selected. Both PZQ and INPs were given orally starting from the 42nd dpi. All animals were sacrificed on the 49th dpi. Parameters used to evaluate INPs therapeutic efficacy in comparison to PZQ included parasitological and histopathological assessments.

Parasite and snails: The Egyptian strain of *S. mansoni* was used in the current study. Twenty shedding adult *B. alexandrina* snails (4-6 mm in diameter) were obtained from the Schistosome Biological Supply Centre, Theodor Bilharz Research Institute, Cairo, Egypt. Snails were allowed to shed under light and the fresh exiting cercariae were used to infect the mice. Each mouse was infected with 100 freshly shed cercariae using the paddling technique[24].

Experimental animals: Forty male Swiss strain albino mice, four to six weeks old, weighing 20-25 grams each were obtained from the animal house of the Medical Parasitology Department, Faculty of Medicine, Alexandria University. Egypt. Mice were kept in a

Table 1. Study groups of mice.

Groups	Characteristics
I	Infected, and non-treated.
II	Infected and treated orally with PZQ in a dose of 500 mg/kg once.
III	Infected and treated with ZV-INPs (10 mg/kg/d) for four consecutive days.
IV	Infected and treated with MNPs (10 mg/kg/d) for four consecutive days.

MNPs: Magnetite INPs; **PZQ:** Praziquantel; **ZV-INPs:** Zero-valent INPs

pathogen-free environment with standard conditions of light and temperature. They were fed on bread and milk in alternation with wheat. The animals had free access to food and water.

Tested agents

PZQ: Distocide TM (EIPICO, Egypt), 600 mg tablets, was purchased from the local pharmacy. One tablet was crushed before use and dissolved in 6 ml 60% ethyl alcohol to obtain a solution with a concentration of 100 mg/ml. On the 42nd dpi, each mouse in group II received 0.1 ml of the prepared solution containing 10 mg of PZQ orally by gavage using a ball-tipped feeding needle (i.e., total dose of 500 mg/kg)[25].

Biosynthesis of INPs: The biogenic synthesis of MNPs and ZV-INPs was performed under aerobic and anaerobic conditions by *Proteus mirabilis* strain 10B as previously described[26,27].

Characterization of INPs: To identify the criteria of the biologically synthesized MNPs and ZV-INPs, the following assessments were conducted[28,29]. First, measurement of absorption spectra was done by UV-Vis spectrophotometer (Labomed model UV-Vis double beam spectrophotometer, USA). Second, determination of the crystalline nature was identified by X-ray diffractometer (Shimadzu 7000, USA). Third, description of the size and morphology was accomplished by transmission electron microscope (JEOL JEM-1230, Japan). Fourth, study of the magnetic features was performed by vibration sample magnetometry (VSM). Finally, measurement of electrostatic potential with polydisperisty index (PDI) was recorded by Zetasizer Nano (Malvern Instruments, Worcestershire, UK).

Administration of INPs: On the 42nd dpi, each mouse in groups III and IV received 0.2 ml of the prepared suspension composed of 1 mg/ml of ZV-INPs and MNPs, respectively. Mice were inoculated orally by gavage using a ball-tipped feeding needle for four consecutive days.

Parasitological assessment: Mice infection was confirmed by stool examination starting from the 35th dpi. The parasitological assessment included the estimation of the total count of adult worms, female worm load, tissue egg count in both liver and intestine, as well as female fecundity. Adult worms were recovered from the hepatic and mesenteric vessels by perfusion technique[30] to assess adult worm burden after mice sacrifice on the 49th dpi[31]. All mice were injected with 500 units of heparin and then anaesthetized by IV injection of an overdose (150 ml/kg) of thiopental sodium[32]. Adult worms recovered from the hepatic and portomesenteric vessels were counted[33]. To determine the tissue egg count, parts of the liver and intestine from each mouse were weighed, cut into small pieces, then artificially digested by 10 ml of 4% potassium hydroxide for each gram of tissue. The containers were covered and left overnight at room temperature to ensure complete tissue digestion without egg destruction. After thorough shaking, eggs present in 0.1 ml of the tissue suspension were counted five times on five separate slides. To determine the number of eggs/one ml of the digestive fluid, the sum of the five readings was multiplied by 20 to obtain the egg count in ten ml of fluid representing the egg count/gram tissue[33]. The mean adult female fecundity was calculated separately for each group. Fecundity of the adult female worms was calculated according to the following equation; fecundity = number of eggs in one gram of intestine/number of adult females[24,34]. Females were identified by a dissecting microscope being longer and thinner than males, with rudimentary suckers and thin cuticle from through which acid heamatin in the intestinal caeca could be detected easily.

Histopathological study: Half of the liver was fixed in 10% formalin and stained with haematoxylin and eosin stain to determine granuloma number and size. Mean granulomata number was determined in ten successive fields of five slides from each mouse, and was accordingly determined in each group[35]. Similarly, mean granuloma size in each mouse was calculated by measuring their diameters under the light microscope, equipped with an ocular micrometer. Only granulomata surrounding eggs were measured. The mean diameter was calculated from ten granulomata, and the mean granuloma size was calculated for each group[36].

Statistical analysis: Data were analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). The Kolmogorov-Smirnov test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). *F*-test (ANOVA) test was used for normally distributed quantitative variables to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons. Kruskal Wallis test was used for abnormally distributed quantitative variables to compare between more than two studied groups, and Post Hoc (Dunn's multiple comparisons test) for pairwise comparisons. Statistical significance was considered when $P \leq 0.05$.

Ethical approval: The experiment was conducted according to the institutional ethical guidelines for animal use in research. The study protocol was approved by the Ethics Committee of Faculty of Medicine, Alexandria University, Egypt. (No 0305191).

RESULTS

Characterization of INPs: The crystalline nature of both INPs was confirmed by X-ray diffraction. Their optical properties were studied by UV-Vis spectroscopy to detect surface plasmon resonance at 410 (MNPs)

and 265 nm (ZV-INPs). Their size and morphology were visualized by transmission electron microscope revealing spherical, tiny, and mono-dispersed INPs with a size range of 1.44–1.92 nm and 11.7–60.8 nm for MNPs and ZV-INPs, respectively. Zeta potential was used for affirming the long-term stability of MNPs and ZV-INPs and recorded -66.4 mV and -31.8, respectively. The higher homogenous dispersity nature of MNPs than ZV-INPs was confirmed by measuring PDI which recorded 0.212 and 0.477, respectively. Furthermore, VSM was used to study the magnetic features of both types of INPs. Results of VSM indicated that MNPs exhibited superparamagnetic behavior by recording 49.5 emu/g, whereas the saturation magnetization of ZV-INPs was1.8 emu/g.

Mortality rates in the study groups: The mortality rate was 50% (5 mice died out of 10) in the non-treated control (group I). There was a decrease in mortality rate of mice in the treated groups: 40% in group III and 30% in groups II and IV.

Adult worm load: Counts of total adult *S. mansoni* worms are presented in table (2). Both PZQ and MNPs showed a statistically significant adult female worm reduction compared to untreated group I and ZV-INPs treated group III, with no significant difference between the former two agents. However, it should be denoted that ZV-INPs also showed a significant ($P \leq 0.05$) female worm load reduction effect compared to untreated group I. For total adult worm load, PZQ-treated group II showed significantly ($P \leq 0.05$) lower total count compared to the control group I, and INPs-treated groups III and IV. In the same respect, MNPs showed a significant ($P \leq 0.05$) reduction in the total adult worm load compared to the control untreated group. On the contrary, ZV-INPs treated group III did not show statistically countable worm load reduction compared to other studied groups.

Tissue egg count: Hepatic and intestinal tissue egg loads are demonstrated in table (3). The INPs in groups III and IV exerted a significant ($P \leq 0.05$) hepatic egg load reduction than in the untreated group I, and insignificant difference between the former two groups or between INPs- and PZQ-treated groups. Moreover, the presently tested INPs showed a nearly equal significant upper hand over PZQ in intestinal tissue egg load reduction, with consequent significance when compared to untreated group.

Adult female worm fecundity: A paradox increase in female fecundity was demonstrated in PZQ treated group II compared to the untreated control However, MNPs had a privilege in this aspect where they showed the significantly lowest female fecundity levels, followed by ZV-INPs (Table 4).

Histopathological results: Results of H&E-stained liver sections of the study groups revealed significant reduction in granuloma number in groups II (PZQ-treated), and IV (MNPs-treated), compared to untreated group I and ZV-INPs treatment in group III ($P \leq 0.05$). The latter did not show any statistical difference compared to the control group (Table 5). However, the mean granuloma size in different groups (Table 6) showed significant diminution in granuloma size using PZQ and ZV-INPs compared to the untreated control group ($P \leq 0.05$). Interestingly, despite the statistical insignificant down-sizing effect of MNPs compared to the untreated group, these NPs showed a reduction in granuloma size approaching that obtained from the use of the other two agents, and consequently recording insignificant difference between the three agents.

Histopathological examinations revealed the typical bilharzial granulomata with abundant epithelioid cells, histiocytes, plasma cells, eosinophils, and fibroblasts, while hepatocytes show marked swelling

Table 2. Comparison between the different study groups according to adult female and total worms count.

Worm burden	Group I	Group II	Group III	Group IV	Significance@
Adult female					
Min.-Max.	22.0 - 38.0	1.0 - 3.0	4.0 - 28.0	3.0 - 10.0	
Mean ± SD	30.7 ± 6.6	2.0 ± 0.9	15 ± 9.0	5.50 ± 2.6	***F* = 29.931***
Median	31.5	2.0	14.0	4.50	***P* < 0.001***
IQR	24.0 - 37.0	1.0 - 3.0	8.0 – 23.0	4.0 - 7.0	
***P*0**	--	<0.001*	<0.001*	<0.001*	
Significance#	--	***P*1 = 0.004*, *P*2 = 0.720, *P*3 = 0.039***			
Total worms					
Min.-Max.	52.0 - 62.0	2.0 - 7.0	30.0 - 69.0	31.0 - 48.0	
Mean ± SD	56.0 ± 4.05	4.2 ± 2.0	49.0 ± 15.0	41.8 ± 6.0	***F* = 45.261***
Median	54.50	4.0	45.0	44.0	***P* < 0.001***
IQR	53.0 - 60.0	2.0 - 6.0	40.0- 65.0	39.0 - 45.0	
***P*0**	--	<0.001*	0.490	0.039*	
Significance#	--	***P*1 < 0.001*, *P*2 < 0.001*, *P*3 = 0.471**			

Group I: Control; **Group II:** PZQ-treated; **Group III:** ZV-INPs-treated; **Group IV:** MNPs-treated. ***F:*** Value for ANOVA test; ***P:*** Value for post-hoc test (pairwise comparison between each two groups). ***P0:*** *P* value for comparing between group I and each of the other groups; ***P1:*** *P* value for comparing between groups II and III. ***P2:*** *P* value for comparing between groups II and IV; ***P3:*** *P* value for comparing between groups III and IV; **IQR**: Interquartile range; **@:** Significance within groups; **#:** Significance between groups; ***:** Statistically significant at $P \leq 0.05$.

(Fig 1a). Treated groups III (Fig. 1b) and IV (Fig. 1c) showed fewer granulomata with amelioration of liver pathology as shown by decreased granuloma size, mild hepatocytes' swelling, and mild inflammatory cellular reaction characterized by lymphocytes abundance and fewer fibroblasts.

Table 3. Comparison between the different study groups according to tissue egg count.

Egg count	Group I	Group II	Group III	Group IV	Significance[@]
Hepatic					
Min.-Max.	190.0 - 402.0	60.0 - 82.0	0.0 - 59.0	1.0 - 36.0	
Mean ± SD	294.8 ± 78.8	70.7 ± 8.0	30.67 ± 19.0	15.2 ± 11.7	***F* = 59.924***
Median	305.0	72.0	30.50	13.0	***P* < 0.001***
IQR	220.0 - 347.0	63.0 - 75.0	27.0 - 37.0	10.0 - 18.0	
***P*0**	--	<0.001*	<0.001*	<0.001*	
Significance[#]	--	***P*1 = 0.358, *P*2 = 0.123, *P*3 = 0.914**			
Intestinal					
Min.-Max.	248.0 - 393.0	70.0 - 100.0	10.0 - 45.0	0.0 - 17.0	
Mean ± SD	342.5 ± 57.7	85.7 ± 11.9	24.8 ± 13.20	8.2 ± 5.9	***F* = 156.64***
Median	357.0	88.0	24.50	7.50	***P* < 0.001***
IQR	310.0 - 390.0	73.0 - 95.0	12.0 - 33.0	5.0 - 12.0	
***P*0**	--	<0.001*	<0.001*	<0.001*	
Significance[#]	--	***P*1 < 0.012*, *P*2 < 0.001*, *P*3 = 0.778**			

Group I: Control; **Group II:** PZQ-treated; **Group III:** ZV-INPs-treated; **Group IV:** MNPs-treated. ***F:*** Value for ANOVA test; ***P:*** Value for post-hoc test (pairwise comparison between each two groups). ***P0:*** *P* value for comparing between group I and each of the other groups; ***P1:*** *P* value for comparing between groups II and III. ***P2:*** *P* value for comparing between groups II and IV; ***P3:*** *P* value for comparing between groups III and IV; **IQR**: Interquartile range; **@:** Significance within groups; **#:** Significance between groups; ***:** Statistically significant at $P \leq 0.05$.

Table 4. Comparison between the different study groups according to worm fecundity.

Worm fecundity	Group I	Group II	Group III	Group IV	Significance[@]
Min.-Max.	10.57 - 1238.0	333.0 - 7300.0	143.4 - 2500.0	0.0 - 180.0	
Mean ± SD	945.7 ± 464.6	4349.8 ± 2587.2	550.5 ± 955.1	99.25 ± 74.9	***F* = 14.499***
Median	1095.9	4150.0	164.9	126.8	***P* < 0.02***
IQR	1034.0 - 1200.0	3166.0 - 7000.0	150.0 - 180.0	12.0 - 150.0	
***P*0**	--	0.094	0.438	0.041*	
Significance[#]	--	***P*1 < 0.014*, *P*2 < 0.001*, *P*3 = 0.205**			

Group I: Control; **Group II:** PZQ-treated; **Group III:** ZV-INPs-treated; **Group IV:** MNPs-treated. ***F:*** Value for ANOVA test; ***P:*** Value for post-hoc test (pairwise comparison between each two groups). ***P0:*** *P* value for comparing between group I and each of the other groups; ***P1:*** *P* value for comparing between groups II and III. ***P2:*** *P* value for comparing between groups II and IV; ***P3:*** *P* value for comparing between groups III and IV; **IQR**: Interquartile range; **@:** Significance within groups; **#:** Significance between groups; ***:** Statistically significant at $P \leq 0.05$.

Table 5. Comparison between the different study groups according to granuloma number.

Granuloma number	Group I	Group II	Group III	Group IV	Significance[@]
Min.-Max.	10.40 - 16.0	6.0 - 7.40	8.0 - 16.0	4.40 - 6.60	
Mean ± SD	13.80 ± 1.9	6.80 ± 0.5	11.1 ± 3.4	5.70 ± 0.90	***F* = 21.134***
Median	14.20	6.80	10.20	5.90	***P* < 0.001***
IQR	13.0 - 15.0	6.40 - 7.20	8.0 - 14.40	5.0 - 6.40	
***P*0**	--	<0.001*	0.134	<0.001*	
Significance[#]	--	***P*1 < 0.06*, *P*2 < 0.797, *P*3 = 0.001***			

Group I: Control; **Group II:** PZQ-treated; **Group III:** ZV-INPs-treated; **Group IV:** MNPs-treated. ***F:*** Value for ANOVA test; ***P:*** Value for post-hoc test (pairwise comparison between each two groups). ***P0:*** *P* value for comparing between group I and each of the other groups; ***P1:*** *P* value for comparing between groups II and III. ***P2:*** *P* value for comparing between groups II and IV; ***P3:*** *P* value for comparing between groups III and IV; **IQR**: Interquartile range; **@:** Significance within groups; **#:** Significance between groups; ***:** Statistically significant at $P \leq 0.05$.

Table 6. Comparison between the different study groups according to size of granuloma.

Granuloma size	Group I	Group II	Group III	Group IV	Significance[@]
Min.-Max.	277.5 - 675.0	285.0 - 360.0	232.5 - 397.5	262.5 - 450.0	
Mean ± SD	407.1 ± 98.2	317.5 ± 31.2	336.7 ± 50.80	362.9 ± 63.96	***F* = 4.169***
Median	412.5	311.0	352.5	367.5	***P* = 0.010***
IQR	330.0 - 450.0	290.0 - 348.0	300.0 - 375.0	300.0 - 412.5	
***P*0**	--	0.048*	0.018*	0.239	
Significance[#]	--	***P*1 < 0.944, *P*2 < 0.555, *P*3 = 0.705**			

Group I: Control; **Group II:** PZQ-treated; **Group III:** ZV-INPs-treated; **Group IV:** MNPs-treated. ***F:*** Value for ANOVA test; ***P:*** Value for post-hoc test (pairwise comparison between each two groups). ***P0:*** *P* value for comparing between group I and each of the other groups; ***P1:*** *P* value for comparing between groups II and III. ***P2:*** *P* value for comparing between groups II and IV; ***P3:*** *P* value for comparing between groups III and IV; **IQR**: Interquartile range; **@:** Significance within groups; **#:** Significance between groups; ***:** Statistically significant at $P \leq 0.05$.

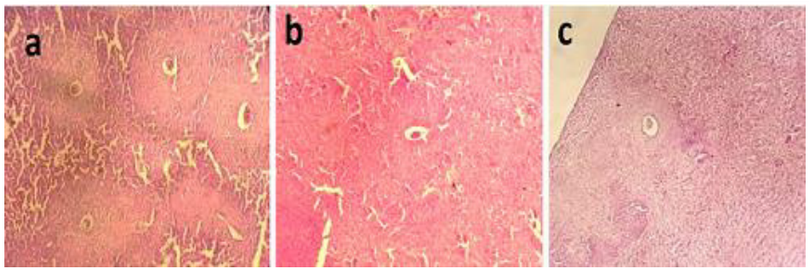

Fig. 1. Infected non-treated group (I) showing multiple typical bilharzial granulomata **(1a)**. Infected ZV-INPs-treated group (III) showing fewer granulomata with amelioration of liver pathology as shown by decreased granuloma size **(1b)**. Infected MNPs-treated group (IV) showing fewer granulomata with preservation of liver architecture **(1c)**. (H&E, x100).

DISCUSSION

Schistosomiasis is an endemic disease of poverty that is related to unhygienic habits and the use of sewage-contaminated water sources. In addition, the continuous migration of infected individuals to other communities introduced the disease to unexpected areas[1]. In the last few decades, controlling programs caused decreased prevalence of schistosomiasis in Egypt, however the disease is still endemic in many sporadic areas[37]. Depending mainly on PZQ treatment of schistosomiasis inferred great challenge in terms of effectiveness and resistance[4].

Nanoscale particles formed by metals are characterized by their physicochemical, electrical, magnetic and thermal privileges, hence, their use in biomedical, agricultural, and environmental fields[38,39]. Being one of the natural metals in the earth's crust, and the fact that it retains essential metabolic roles in all living organisms, iron and consequently INPs, are more likely to be biocompatible and safer than other compounds used in metallo-pharmaceuticals[40,41]. Thus, some INP formulations have recently gained approval from the Food and Drug Administration for use as therapeutic agents as well as indispensable chaperones in modern diagnostics[42]. It is worth mentioning that nanotechnology strived to find more environment-friendly production approaches for NPs. Biosynthesis of NPs is regarded to be a more eco-friendly, non-toxic, and cost-effective process when compared to traditional methods. The biogenic synthesis of both MNPs and ZV-INPs exhibited antagonistic activity against a broad spectrum of examined microbial pathogens (bacteria, biofilm, yeast and algae)[43].

Both biosynthesized INPs prepared in the current study had a uniform shape as shown by ultrastructural characterization, ruling out artefactual and necrotic bodies[44,45]. The highly negative zeta potential confers INPs dispersion in solution and resistance to aggregation, that was also confirmed in their uniform spherical shape[46]. The homogenous nature of the solution used is again confirmed by the PDI values that were less than 1 in the present work[47]. Besides, VSM characterization proved the superparamagnetic properties for MNPs and not for the ZV type that was even less than 2 nm. In agreement Bean and Livingston[48] had stated that super-paramagnetism arises when the diameter of the magnetic material is below a certain value and typically when it is between 3 and 50 nm. Conversely, toxicological study of ZV-INPs indicated no evidence of liver damage as well as no significant change in the level of serum amylase and lipase activities following acute oral exposure[49]. Additionally, satisfactory toxicological profiles with no clinically significant side effects have been reported for iron oxide NPs according to the standard pharmacological tests following oral administration[50].

In the present study, although PZQ showed a powerful impact on adult worm load reduction, results showed that MNPs in groups III and IV are superior to PZQ as regards lowering adult female worms' fecundity. This led to significant decrease in tissue egg counts in groups III and IV compared to untreated group I (liver and intestine) and PZQ-treated group II (intestine), with insignificant difference between both INPs types. As regards the histopathological results presented in the study, the effect of both INPs approached that of PZQ in the amelioration of liver pathology as observed by reduction of granuloma number (ZV-INPs) and size (MNPs).

Comparing between INPs types, the MNPs showed privilege over ZV-INPs in female and total adult worm reduction, and in lowering liver granulomata number. This is consistent with Zaki *et al.*[45] who studied antimicrobial effect of INPs and observed that MNPs were more efficient than ZV-INPs at suppression of microbial growth in all examined water samples due to their smaller size, and hence, their superparamagnetic properties with the release of hyper reactive oxygen species[45]. Despite the present results that showed ineffectiveness of MNPs compared to the ZV-INPs in granuloma downsizing, the former had a comparable effect to the latter in the amelioration of liver pathology.

It was proved that INPs uptake by cancer cells can lead to programmed cell death due to iron ion release, leading to more and more production of reactive oxygen species through Fenton's reaction[51,52], and that these steps are blocked from the beginning in normal cells. This anti-cancer effect was explained by the fact that INPs are easily translocated into acidic microenvironments, specifically demarcated by the abundance of lysosomal compartments, as that for

cancer cells, and hence the release of iron ions[53]. Similarly, the gut lumen of *Schistosoma* adult worm has a pH ~4.5[54], because lysosomal secretions are the mainstay feature of the schistosome gastrodermis[55]. Earlier, it was documented that eggshell precursor proteins are synthesized in the vitelline cells[56]. These precursor proteins are packed into large vesicles inside the vitelline cells[57] containing the eggshell precursor emulsion; and the acidic pH of these vesicles is important to stabilize the emulsion and prevent the eggshell cross-linking reactions[58].

Accordingly, the ultimate reduction in adult female worm fecundity observed in INPs treated groups III and IV, can be explained by the inevitable translocation of the NPs into the acidic environment of adult worm gut concerned with digestion of ingested blood, and into the vitelline cells' acidic secretory vesicles. This translocation, sequentially releasing iron ions and super reactive oxygen species, can lead to interruption of hemoglobin digestion, hence nutritional derangements, that in turn can interfere with oogenesis[53]. It can be deduced that similar effects will occur in vitellocytes' vesicular compartments of adult female schistosomes, leading to failure of eggshell development. Similarly, and on the same basis, we can assume the potential effectiveness of the tested NPs in reduction of tissue egg load and granuloma number, that approached and even exceeded PZQ efficacy. It was reported that ZV-INPs had higher ability to release free ions in acidic environment and thus it could be expected that they might have advantage over MNPs in their antischistosomal effect[52].

Aside from the mechanism by which normal cells are not damaged by INPs treatments for cancer, and that cancer cells' behavior differs extremely from normal cells, here we can confirm that MNPs do not damage the surrounding host's environment as previously described[59]. That is, the excess intracellular breakdown products of these INPs are sequestered in intracellular ferritin stores, hence limiting exaggerated toxic oxidative response. It was noticed that elevated serum ferritin is an inflammatory marker that indicates disease progression, and is a feature of hemophagocytic lymphohistiocytosis which is a known complication to infection, and is closely related to poor prognosis in corona virus disease[60]. The paradox increase of fecundity in PZQ treated mice could be attributed to the significant effect of the drug on the adult female worm burden that gave false result while dividing the tissue eggs on the bare number of the live females.

It was reported that the introduction of MNPs has its effect on macrophage polarization and inflammatory outcome[61]. A shift towards type 1 macrophages was observed with the production of pro-inflammatory cytokines, e.g., interleukin-12, exemplifying the immunomodulatory effect of iron oxide[62,63]. Interleukin-12 is known to stimulate T-helper 1 lymphocytes differentiation and interferon gamma production which are important in protection against severe schistosomal pathology[64,65]. The present work, in contrast, showed that ZV-INPs, and not MNPs, simulated PZQ in down-sizing hepatic granulomata. This means that even introducing ZV-INPs can ameliorate *Schistosoma* induced liver pathology by manipulating inflammatory cell reaction towards T helper 1 type.

Taking in consideration that amelioration of schistosomiasis-induced liver pathology is an indispensable therapeutic goal to decrease the risks of portal hypertension and liver derangements; we can conclude that INPs in the two forms used in our study are potentially effective anti-schistosomal agents. To our knowledge, this is the first study to prove the indigenous anti-schistosomal activity of INPs *in vivo* as shown by decreasing adult female worm fecundity and hence reduction of tissue egg load and liver granulomata number, together with down-sizing and inflammatory cell-type transformation of hepatic granulomata. Finally, further studies are still required to elucidate the action mechanisms of both INPs in schistosomiasis. Additionally, the effect of cellular uptake of INPs on levels of serum ferritin in case of schistosomiasis needs to be further elucidated. Regarding NPs effects on hepatic squeals compared to PZQ, we recommend extension of the experiment duration to 8 or 10 weeks to investigate any new agents regarding the effect on bilharzia hepatic fibrosis.

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