Molluscicidal and Antioxidant Activities of Silver Nanoparticles on the Snail Intermediate Hosts of Schistosomiasis

Khaled M. Zayed (✉ khaled.z1@yahoo.com)

Theodor Bilharz Research Institute <https://orcid.org/0000-0002-5026-0084>

Yun-Hai Guo

National Institute of Parasitic Diseases

Xiao-Nong Zhou

National Institute of Parasitic Diseases

Research

Keywords: Schistosomiasis, *B. alexandrina*, *B. glabrata*, *Oncomelania*, silver nanoparticles, *D. magna*, antioxidants.

Posted Date: July 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-718810/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Schistosomiasis, also known as bilharzia, is a disease caused by parasitic worms. This disease is the second important parasitic disease after malaria in terms of its burden of disease.

Methods: One of control intervention for schistosomiasis control programme is to perform the mollusciciding to interrupt the transmission through snail intermediate host where the parasites developed from miracidia to cercaria that is the infective stage. Accordingly, it is possible to improve the mollusciciding effects after adding nanoparticulate agents in molluscicides.

Results: This research field has caused more attention, since silver nanoparticles (Ag-NPs) were characterized by the transmission electron micrographs (TEM) with X-Ray fluorescence (XRF), showed that it's mostly spherical in shape with average particle size diameters 20nm with high purity of synthesized particles (99.99%) of element was Ag. The aim of the present study is to demonstrate the molluscicidal effects of Ag-NPs against the three species of snails, including two species of intermediate snail host of *Schistosoma mansoni* (*Biomphalaria alexandrina* and *Biomphalaria glabrata*) and one species of intermediate snail host of *S. japonicum* (*Oncomelania hupensis*), and the cercaricidal effects of Ag-NPs against *Schistosoma mansoni* cercariae. At the same time, their effects on snail's antioxidant enzymes, e.g. catalase (CAT), glutathione reduced (GSH), nitric oxide (NO) and total antioxidants capacity (TAC), and on freshwater cladoceran (crustacean) *Daphnia magna* that represent the potential toxicity to other aquatic organisms were observed as well. The results indicated that Ag-NPs had a molluscicidal effect on tested snails with the variation in lethal concentration dose. LC₅₀ of Ag-NPs exposure for 24, 48, 72 hrs and 7 days to *B. alexandrina* snails was 7.91, 5.69, 3.83 and 1.91 ppm respectively, LC₅₀ for 24, 48, 72 hrs and 7 days exposure to *B. glabrata* snails was 16.55, 10.44, 6.91 and 4.13 ppm respectively, while LC₅₀ for 24, 48, 72 hrs and 7 days exposure to *Oncomelania hupensis* snails was 46.5, 29.85, 24.49 and 9.62 ppm respectively. While the cercaricidal effects on *S. mansoni* cercariae was fast, since the lethal time LT₅₀ and LT₉₀ exposed to Ag-NP with 80 ppm concentration was 27 min and 45 min, respectively; and its 100 ppm exposed was 15min and 41 min, respectively. Moreover, there is no mortality rate to *Daphnia magna* when exposed to more than double and half dose (50 ppm) of Ag-NPs for continuous 3 hs, of which LC₉₀ to *B. alexandrina* snails was 18 ppm.

Conclusions: The synergistic effect of synthesized Ag-NPs as molluscicidal and cercaricidal was clearly revealed that it's promising to be effectively used against various intermediate host snails of intestinal schistosomes and reduced it's a potential antioxidant activity.

Background

Schistosomiasis is infectious disease transmitted through snails because of the infection with a blood fluke of the genus *Schistosoma*. Two forms of the disease distributed in the 78 countries of the world, including urogenital disease which is caused by *Schistosoma haematobium* infection, and the intestinal form caused mainly by *S. mansoni*, *S. japonicum*, *S. intercalatum*, and *S. mekongi* [1]. While two forms, *S.*

mansoni, *S. japonicum*, are mostly threatening to human health in the tropics. With the progress of the national schistosomiasis control programmes, the disease prevalence is getting down and protection from schistosome infection is more important than treatment only. Therefore, it is an increasing need for effective control procedures in the national schistosomiasis control programmes [2]. Two intestinal species that infect human beings, including *S. mansoni* and *S. japonicum*, are transmitted by intermediate host snails associated with genera *Biomphalaria* and *Oncomelania*, respectively. *Biomphalaria alexandrina*, the intermediate host of *S. mansoni*, is widely distributed in Egypt [3], *B. glabrata*, responsible for *S. mansoni* transmission, is distributed in many countries of South America [4], and *O. hupensis* is an intermediate host of *S. japonicum* distributed in China, Japan, Philippines and Indonesian island of Sulawesi [5].

Egypt is the first country around the world to initiate national schistosomiasis control programme. Since 1922, the national schistosomiasis control programme has been on the schedule for the Ministry of Health and Population in Egypt [6]. Various control strategies had been followed. Transmission control strategy was followed pursuant to WHO recommendations that targeted the control of schistosomiasis transmission by reducing its snail intermediate host along with anti-schistosomal medication of infected cases. In terms of mollusciciding, as one of important components for the national schistosomiasis control programme, different mollusciciding approaches have been tried in different stage. For example, in 1922, a national attack was launched against snails together with the treatment of some seriously infected persons in Egypt. In 1926, the first planned control programme was adopted at Dakhla oasis through using molluscicide, or copper sulfate, to all irrigation canals [7]. In 1961, molluscicides were used in transmission sites, areas of land rehabilitation and in areas in which seasonal irrigation is changed into perennial irrigation. The molluscicides including copper sulfate, sodium pentachlorophenate and niclosamide were implemented in the snail ridden fields. All those drugs had serious side-effects to those patients during and after treatment [7]. It should also worth noted that the application of molluscicides is a procedure that is not only highly cost in application and necessary to be supported by great funds, but also is a repeat process for years to prevent snail from getting back into water courses again [8], besides the application of molluscicides can be toxic to environment. Therefore, more cost-effectiveness approaches were explored, including lining of canals with concrete to protect against the formation of snail colonies [9]. In, Egypt, plant molluscicides have been also applied in recent years [10]. In addition, use of natural carnivores, such as fish (*Tilapia* spp.), ducks and aquatic insects, which can play as natural enemies had been used as a biological control [2].

On the other hand, nanotechnology is used to modify materials at the nano-scale (< 100 nm) to create narrative properties. Changes in the physicochemical and structural properties of materials that resulted from the reduction in particle size can lead to new and sometimes unexpected biological effects [2]. The synthesis of metallic nanoparticles has become lately an active field of researches, and more importantly in nanotechnology. Much attention has been paid to metallic nanoparticles because of their extraordinary physical and chemical properties, which broadly differ from their bulk properties [11]. Moreover, several studies analyzed the antibacterial mechanisms of action of Ag-NPs on which numerous factors, such as NPs diameter, shape, surface changes, etc., has a great influence on the efficacy of molluscicides.

Furthermore, nanoparticles are extensively used in the manufacture of textiles, materials to dispose of microorganisms and the clothes odor, food packaging, paints, cosmetics and biomedical products, mainly where antimicrobial properties are coveted [12]. By advantage usage of silver and its antimicrobial properties, it is essential to investigate the toxic probability effects of Ag-NPs against different organisms and non-target species.

Previous studies have showed that molluscicides broadly have oxidative stress effect on the snails. It is well known that oxidative stress occurs in organisms when the generation of oxygen radicals rate become more than the rate of their decomposition [13]. Among the essential antioxidants parameters, not so many of antioxidants were presented in the snails that had effect on the oxidative stress. Those antioxidants are total antioxidants capacity (TAC), glutathione reduced (GSH), nitric oxide (NO) and catalase (CAT), where TAC is the marker that evaluates the antioxidant status of the biological system and is very useful to estimate the response of the organism against the free radical production [13]. Reduced GSH is the first line of non-enzymatic antioxidant of the defense system that attack the oxidative stress and gets depleted in the oxidative stress models [14]. CAT is the enzyme that changes superoxide into water and molecular oxygen and prevents the creation of hydroxyl radical and other toxic ROS species [15]. It is also worth to mention that most of ecotoxicological tests on hazardous materials using invertebrates had been conducted using crustaceans. Regarding ecotoxicological tests on manufactured nanomaterials, most tests have used the same species as testing organism, such as *Daphnia magna* [16].

The present study aimed to evaluate the molluscicidal effects of silver nanoparticles (Ag-NPs) on three species of snails, including *B. alexandrina*, *B. glabrata*, both species are intermediate snail hosts of *S. masoni*, and *O. hupensis*, the intermediate snail host of *S. japonicum*, and in vitro cercaricidal effect on *S. mansoni* cercariae. At the same time, the potential molluscicidal effectiveness mechanism as well as the assessment their effects on the other aquatic organisms, *Daphnia magna*, were explored.

Materials And Methods

Snails:

B. alexandrina, *B. glabrata* and *O. hupensis* snails were obtained from both Egyptian laboratory stock at Malacology Department, Theodor Bilharzias Institute based in Caro of Egypt, and National Institute of Parasitic Diseases, Chinese Center for Diseases Control and Prevention based in Shanghai of China.

B. alexandrina and *B. glabrata* snails were maintained, as stock cultures, in a well-prepared snail room, under suitable environmental conditions, in glass aquaria containing dechlorinated tap water in a density of 10 snails / L. The snails were fed on fresh lettuce leaves, supplemented with tetramine (fish food) and chalk, after careful selection on the basis of size and age. .

O. hupensis, breeding system for *O. hupensis* consists essentially of an aquaterrarium for adult breeding snails and an aquarium for juvenile snails. The water in the aquarium connected with that in the terrarium

through a pumping tube and a drain pipe. Newly hatched snails and egg masses are flushed into the aquarium by flooding the aquaterrarium once a fortnight. Normally the water level of the terrarium is kept just below the surface of the mud. When young snails reach a size of more than 3 mm in shell height they are moved to the aquaterrarium. Optimum area per snail was found to be 40 cm²/ snail for breeding adults and 10 cm²/ snail for growing juveniles. The optimum light intensity for breeding seemed to be produced by one 20 W 60 cm long vegetable fluorescent tube at a distance of 15 cm above the aquaterrarium. Both adult and juvenile snails are fed a modified Standen alginate food including dehydroacetic acid as an antiseptic. [17]

Hemolymph:

Snail hemolymph was collected using the techniques described by [18]. The hemolymph was obtained via a small hole made in the shell into which capillary tube was inserted then it was drawn into tube by capillary suction, collected sample tested fresh after drawn from the snails.

Enzyme assays:

Total antioxidants capacity (TAC)

The determination of the antioxidative capacity is performed by the reaction of antioxidants in the sample with a defined amount of exogenously provide hydrogen peroxide (H₂O₂). The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H₂O₂ is determined colorimetrically by an enzymatic reaction which involves the conversion of 3,5-dichloro - 2- hydroxy benzensulphonate to a colored product [19].

Glutathione reduced (GSH)

The method based on the reduction of 5,5` dithiobis (2 - nitrobenzoic acid) (DTNB) with glutathione (GSH) to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm [19].

Catalase (CAT)

The enzyme catalyzes the detoxification of H₂O₂ produced after the dismutation of O₂ - by SOD. It was assayed according to Lubinsky and Bewley (1979) using 10 mM H₂O₂. The decrease in absorbance was read at 230 nm and the activity of the enzyme was calculated using an extinction coefficient 25 for the enzyme. α-Glycerophosphate dehydrogenase activity was assayed using d-glyceraldehyde 3-phosphate as substrate (10 mM) and NAD (15 mM) in 0.1 M phosphate buffer, pH 7.0 [20]. The increase in absorbance was measured at 340 nm.

Nitric Oxide (NO) is synthesized in biological system by the enzyme Nitric Oxide Synthase (NOS). NOS is a remarkably complex enzyme which acts on molecular oxygen, arginine, and NADPH to produce NO, citrulline, and NADP⁺. NOS O₂⁺ Arginine⁺ NADPH. NO⁺ Citrulline⁺ NADP NO is produced in trace quantities

by neurons, endothelial cells, platelets, and neutrophils in response to homeostatic stimuli. This NO is scavenged rapidly ($t_{1/2} = 4$ seconds) and acts in a paracrine fashion to transduce cellular signals. NO is also produced by other cells (macrophages, fibroblasts, hepatocytes) in micromolar concentrations in response to inflammatory or mitogenic stimuli. The final products of NO in vivo are nitrite (NO_2^-) and nitrate (NO_3^-). The relative proportion of NO_2^- and NO_3^- produced from NO is variable. The exogenous source of NO_3^- ingested in the diet should be considered and cannot be ignored (non-NO origin). Thus, one of the indexes of NO production is the NO_2^- . The Biodiagnostic Nitrite Assay Kit provides an accurate and convenient method for measurement of endogenous nitrite concentration as an indicator of nitric oxide production in biological fluids. It depends on the addition of Griess Reagents which convert nitrite into a deep purple azo compound, photometric measurement of the absorbance due to this azo chromophore accurately determines NO_2^- concentration [21].

Silver nanoparticles

Silver Nano Powder (Ag-NPs), Diameter 20 nm with spherical in shape, Content 99.9%, SSA 10–20 m²/g, Density 0.25–0.6 g/cm³, Storage Stored at RT. was purchased from Abvigen company, Newark, USA.

Detection of molluscicidal rate

A serial dilution from Ag NPs was prepared in dH₂O [22]. For each concentration, 10 snails were used (each snail 6–8 mm in diameter) and this was repeated in triplicates. Each snail was exposed to each concentration for 24 h, 48 h and 72 h then allowed to recover for the next 24 h. The result was documented on the next day of recovery; all were maintained at $25^\circ \pm 1^\circ\text{C}$ and pH 7.4. Control snails were maintained under the same experimental conditions in dechlorinated water [23]. The dose 100 µg/ml was chosen empirically as a starting dose; it showed a 100% lethal effect with Ag NPs. Snails were considered dead if they did not move and showed a discolored body retracted into the shell [24]. Percentage of snail mortality was detected and the LC_{50} and LC_{90} values of Ag NPs were determined.

Statistical analysis

Data were analyzed using Statistical Program for Social Science (SPSS) version 20.0 to calculate the LC_{50} and LC_{90} by probit analysis. Analytical statistics included a one-way analysis of variance (F) (ANOVA), Chi-square test of significance, and Post Hoc test. The confidence interval was set to 95% and the margin of error accepted was set to 5%. Probability (*P* value) was calculated based on following criteria: *P* value < 0.05 was considered significant, *P* value < 0.001 was considered as highly significant, and *P* value > 0.05 was considered insignificant.

Results

Characters of silver oxide nanoparticles:

The TEM images showed that silver nano powder nanoparticles were mostly spherical in shape forming chain like aggregates with average particle size diameters 20nm. Elemental analysis of silver nanoparticles was performed using X-Ray fluorescence (XRF) revealed high purity of synthesized particles; 99.99% of element of composition nanoparticles was Ag, specific surface area (SSA) 10–20 m²/g and Density 0.25–0.6 g/cm³ (Fig. 1).

Toxicity test:

Data presented in Table (1) show values of LC₅₀ for 24, 48, 72 hrs and 7 days against *B. alexandrina* snails treated with tested Silver NPs at concentrations of 7.91, 5.69, 3.83 and 1.91 ppm, respectively. While, Table (2) represent the values of LC₅₀ for 24, 48, 72 hrs and 7 days against *B. glabrata* snails treated with Ag-NPs at concentrations of 16.55, 10.44, 6.91 and 4.13 ppm, respectively. Furthermore, in Table (3) show values of LC₅₀ for 24, 48, 72 hrs and 7 days against *O. hupensis* snails treated with Ag-NPs at concentrations of 46.5, 29.85, 24.49 and 9.62 ppm, respectively. While focusing on the variation exposure effect of Ag-NPs on the three species of snails, we find that the molluscicidal effect of Ag-NPs in *B. alexandrina* was more than double effects than that in *B. glabrata* (2.09 and 2.16 times for 24 and 7 days exposure respectively), and there were more than quadrat effect when compared to that in *O. hupensis* (5.87 and 5.03 times for 24 and 7 days exposure respectively). While when we compare between *B. glabrata* and *O. hupensis*, *B. glabrata* has more than double effect (2.8 and 2.32 times for 24 and 7 days exposure respectively).

The current data indicated that the cercaricidal effects for both lethal time LT₅₀ and LT₉₀ exposed to Ag-NP with 80 ppm concentration was (27 min and 45 min., respectively) compared to 100 ppm concentration (15min and 41 min., respectively, Table 4 and Fig. 2). Also, there is no mortality rate to *Daphnia magna* that exposed to more than Double and half dose (50 ppm) of Ag-NPs (for ¼ hour, ½ hr, ¾ hr, 1hr, 2hrs and 3hrs, respectively) which made LC₉₀ (18 ppm) to *B. alexandarina* snails.

Table 1
Molluscicidal activity of the silver nanoparticles (Ag-NPs) against adult *Biomphalaria alexandrina* snails

Exposure time	LC ₁₀ ppm (95% CL)	LC ₂₅ (95% CL) Ppm	LC ₅₀ (95% CL) ppm	LC ₉₀ (95% CL) ppm
24 heure	0.42 (0.17–3.24)	3.04 (1.05–5.43)	7.91 (5.52–10.44)	17.16 (13.79–24.19)
48 heure	0.22 (0.023–2.76)	0.569 (.34– 3.39)	5.698 (2.73–8.24)	15.44 (12.15–22.08)
72 heure	0.15 (.011 – 1.5)	0.383 (0.03–3.1)	3.83 (0.326–6.36)	13.54 (10.3–20.6)
7 days	0.073 (0.003-0.1)	0.183 (0.02–1.37)	1.91 (0.33–3.1)	5.1 (3.7–10.8)

Table 2
Molluscicidal activity of the silver nanoparticles (Ag-NPs) against adult *Biomphalaria glabrata* snail

Exposure time	LC ₁₀ (95% CL) Ppm	LC ₂₅ (95% CL) Ppm	LC ₅₀ (95% CL) Ppm	LC ₉₀ (95% CL) Ppm
24 heure	2.8 (1.4–7.6)	7.02 (0.35–10.9)	16.55 (12.8–20.8)	34.64 (28.3–47.6)
48 heure	1.26 (.98 – 4.6)	3.16 (0.22–8.04)	10.44 (7.02–13.4)	24.25 (20.2–31.1)
72 heure	0.17 (0.09–2.3)	0.437 (0.12–3.88)	6.91 (3.35–9.7)	19.21 (15.7–25.2)
7Day	0.27 (0.02–1.4)	0.685 (0.11–4.5)	4.13 (1.9–6.1)	10.65 (8.2–16.1)

Table 3
Molluscicidal activity of silver nanoparticles (Ag-NPs) against adult *Oncomelania hupensis* Snail

Compound	LC10 (95% CL)ppm	LC25 (95% CL) ppm	LC50 (95% CL) ppm	LC90 (95% CL) ppm
24 heure	7.20 (3.7–17.2)	25.83 (15.3–34.4)	46.52 (37.6–60.1)	85.84 (69.3–108.1)
48 heure	8.96 (2.3–15.1)	18.86 (11.4–23.5)	29.85 (25.1–39.1)	50.74 (40.8–78.4)
72 heure	5.37 (1.6–11.6)	14.43 (5.9–18.8)	24.49 (20.2–30.2)	43.62 (35.8–63.6)
7Day	0.21 (0.02–3.5)	0.50 (.02–5.5)	9.62 (4.2–14.1)	26.96 (20.9–40.5)

Table 4
Toxicity of silver nanoparticles (Ag-NPS) to *schistosoma mansoni* cercariae (lethal time/minutes)

Concentration	LT ₁₀ (95% CL) ppm	LT ₂₅ (95% CL) ppm	LT ₅₀ (95% CL) ppm	LT ₉₀ (95% CL) ppm
80 ppm	8.3 (1.4–18.9)	17.22 (.35–25.9)	27.05 (14.9–36.0)	45.72 (36.6–75.6)
100 ppm	1.12 (.98 – 4.6)	2.11 (.22–7.94)	15.81 (10.52–19.6)	41.8 (37.9–47.2)
LT = Lethal time				

Antioxidant:

The present results are as follows, *B. alexandrina* snail that had exposed to sub-lethal concentration (LC₂₅) of Ag-NPs for three consecutive days showed a high significant decreasing in catalase (3.86 ± 0.015 , 3.85 ± 0.04 and 0.50 ± 0.07 , respectively) than control (28.100 ± 5.89), where there was a high significant decreasing in GSH on the first day (6.43 ± 0.46), and a significant decreasing on the other two days (144.84 ± 1.16 and 139.84 ± 1.49 , respectively) than control (149.14 ± 1.96). In contrast, there was a significant decreasing in TAC on the first day (175.07 ± 2.19), and a high significant decreasing on the other two days (68.98 ± 0.74 and 66.93 ± 2.02 , respectively) than control (187 ± 1.45). On the other hand, there was a high significant decreasing in Nitric oxide assay (NO) on the first two days (0.143 ± 0.011 ,

0.16 ± 0.062, respectively), and a significant decreasing on the third day (0.320 ± 0.01) than control (0.347 ± 0.015, Table 5).

Table 5

Antioxidant parameters of *Biomphalaria. alexandrina* snails after 24, 48 and 72 hours of exposure to LC₂₅ silver nanoparticles (Ag-NPS)

Antioxidant	Control	24h	48h	72h
Catalase assay (U/ L)	28.100 ± 5.890	**3.863 ± 0.015	**3.857 ± .040	**0.503 ± 0.074
Glutathione reduced (GSH), (mg/dl)	149.14 ± 1.96	**6.43 ± 0.46	*144.84 ± 1.16	*139.84 ± 1.49
Total antioxidant capacity (mM/L)	187.82 ± 1.45	*175.07 ± 2.19	**68.98 ± 0.74	**66.93 ± 2.02
Nitric oxide assay (mM/L)	0.347 ± 0.015	**0.143 ± 0.011	**0.160 ± 0.062	*0.320 ± 0.010
* P < 0.05, ** P < 0.01 and Data expressed as Mean ± SD				

Discussion

In this study, Ag-NPs was selected and identified by TEM to appear spherical in shape, separated and not aggregated with a size range of 20 nm in diameter. This size of Ag-NPs proved a potential ability to adhere and absorbed by the surface of target agents that inducing mortality of snail intermediate hosts of schistosomes, this finding was similar to [25] who considered that surface properties of NPs are of essential importance for their aggregation behavior, behind toxicity of Ag-NPs, due to the free silver ions that came from NPs. The antimicrobial properties of ionic silver have known for so long, the complexities faced in various environments coupled with the unique properties and behaviors of NPs have complicated the evaluation of the fate of released Ag-NPs and their organismal interactions [26]. In the presented study, tested Ag-NPs had both molluscicidal and cercaricidal effects, with variation in the lethal concentration dose between them. In the experiment, *B. alexandrina* had the most sensitive molluscicidal effect for Ag-NPs than other species, followed by *B. glabrata* and *O. hupensis*, while *O. hupensis* had the most durable effect during the exposure to Ag-NPs, this finding has an identical with previous reports [27, 28], indicating that Ag-NPs may take actions due to the fact that the creation of free radicals produced from the nanoparticles could discomfort the membrane lipids and then finally spoil the membrane functions [29, 30]. So that our experimental results have reached a new finding that the membrane could be worsened by the creation of pits on the surface of the cell wall membrane, the creation of pits on the membrane resulted in an increase in the permeability and irregular transport that caused the death of the cells. Besides, [31] observed that all *Oncomelania* snails were dead after 144 h under the condition of SNP existence [2], which revealed that Ag-NPs have molluscicidal activity against *B. alexandrina* and cercaricidal in a concentration-dependent manner. In addition, the great impact of Ag-NPs on cercariae was due to Ag ions, which are unceasingly released in water suspension and can bind

to the papillary sites on the cercarial surface, thereby, inhibiting fatty acid-induced cercarial acetabular gland secretion [32].

One of the most widespread and sensitive species used for chemical and NP toxicity screening are the microcrustacean *Daphnia magna* [33–35]. *Daphnia* recreate parthenogenetically facilitating their application as a central toxicological model and indicator species for determining water quality. The present study illustrated that there is no mortality rate to *Daphnia magna* that exposed to more than double and half dose of Ag-NPs for 3 hours continuous exposure, which cause 100% mortality to *B. alexandarina* snails, this indicated the safety to non-target animals by using Ag-NPs as molluscicide in lower doses, this finding has identical with previous reports [36] that uncoated Ag-NPs had a total 100% survival rate of *Daphnia magna* for the first generation. Furthermore, [37] also found that the bioaccumulation of Ag-NPs in the daphnia was lower in the surface water, possibly as a result the heavier aggregation as comparison with that in the a culture M4 medium, could primarily be due to a toxicity which is lower in the surface water.

Several studies have investigated the mechanism of toxicity of Ag –NPs that their cytotoxicity is chiefly mediated by induction of reactive oxygen species (ROS). In this study the antioxidant balances was evaluated by determining the levels of TAC, GSH, NO and CAT, there were a decreasing in all antioxidant enzymes for *B. alexandarina* when exposed sub-lethal concentration of Ag-NPs, of which the finding similar to report of [36] that Ag-NPs considerably inhibited the enzymatic activity of CAT and SOD, announcing that Ag-NPs interacted with them and formed Ag-NP–protein complexes, these results furnished the light on the fact that AgNP–antioxidants interactions exist and modify the secondary structural elements of them. CAT is formed from four identical subunits, which work together to perform its biological function in organisms. In order to reach the active center of CAT, H₂O₂ needs to diffuse inside a narrow channel made of 14 amino acids, inside this channel. The decrease in the activity of CAT could also be illustrated by the interaction of Ag⁺ ions released by Ag-NPs, and by amino acid residues within this narrow channel [38] impeding the approach of H₂O₂ to the active center and decreasing the soluble CAT activity [31].

Conclusion

The presented study demonstrated that the potential applications of silver nanoparticles Ag-NPs in schistosomiasis control because of molluscicidal capability against three types of snails that represent the major intermediate snail hosts for *Schistosoma mansoni* and *Schistosoma japonicum* parasites. The lethal concentration of Ag-NPs varied against three species of snails and schistosome cercaria in vitro, which caused no toxicity to the other aquatic organisms. Therefore, it may become a new, efficient molluscicide for extensive application. The experiments on antioxidant enzymes (TAC, GSH, NO and CAT) interacted with Ag-NPs formed complexes, indicating that the interaction between these enzymes and Ag-NPs is strong enough to promote conformational changes in the enzyme reflecting the snail's immunity.

Abbreviations

Ag-NPs: Silver nanoparticles; CAT: Catalase; DTNB: 5,5` dithiobis (2 - nitrobenzoic acid); GSH: Glutathione reduced; LC: Lethal concentration; LT: Lethal time; NADPH: Nicotinamide adenine dinucleotide phosphate; NO_3^- : Nitrate; NO: Nitric oxide; NOS: Nitric Oxide Synthase; NO_2^- : Nitrite; SNP: Single-nucleotide polymorphism; SPSS: Statistical Program for Social Science; TAC: Total antioxidants capacity; TEM: Transmission electron micrographs; XRF: X-Ray fluorescence.

Declarations

Ethics approval and consent to participate

Ethical clearance of this pilot had been granted approval by the Ethics Committee of the National Institute of Parasitic Diseases, Chinese Center for Disease Control and Prevention in Shanghai, China.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

We declare that we have no competing interests.

Funding

This project was supported by CB-1603/Research Fund for the Technical Reserves, National Institute of Parasitic Diseases, Chinese CDC, and by Young excellent scientists programme of Ministry of Science and Technology of the People's Republic of China.

References

1. STOTHARD JR, Chitsulo L, Kristensen TK, Utzinger J. Control of schistosomiasis in sub-Saharan Africa: progress made, new opportunities and remaining challenges. *Parasitology*. 2009;136:1665–75.
2. Moustafa MA, Mossalem HS, Sarhan RM, Abdel-Rahman AA, Hassan EM. The potential effects of silver and gold nanoparticles as molluscicides and cercaricides on *Schistosoma mansoni*. *Parasitol Res*. 2018;117:3867–80.

3. A El-Emam M, A Gawish F, R Habib M, Wang W, Sun L-P, Engels D, et al. Semi-field and field trials to control *Biomphalaria alexandrina* and *Bulinus truncatus*, intermediate host snails of schistosomiasis in Egypt by the plant molluscicide Luowei/TDS 4%. *Egypt J Aquat Biol Fish*. 2020;24:95–109.
4. Allan ER, Tennessen JA, Bollmann SR, Hanington PC, Bayne CJ, Blouin MS. Schistosome infectivity in the snail, *Biomphalaria glabrata*, is partially dependent on the expression of Grctm6, a Guadeloupe Resistance Complex protein. *PLoS Negl Trop Dis*. 2017;11:e0005362.
5. Ren X, Meng X, Chen D, Tang F, Jiao J. Using silver nanoparticle to enhance current response of biosensor. *Biosens Bioelectron*. 2005;21:433–7.
6. Abou-El-Naga IF. Towards elimination of schistosomiasis after 5000 years of endemicity in Egypt. *Acta Trop*. 2018;181:112–21.
7. Barakat RM. Epidemiology of schistosomiasis in Egypt: travel through time. *J Adv Res*. 2013;4:425–32.
8. Ayad N. The use of molluscicides in Egypt. *Bull World Health Organ*. 1961;25:712.
9. Abou-El-Naga IF. *Biomphalaria alexandrina* in Egypt: Past, present and future. *J Biosci*. 2013;38:665–72.
10. El-Sawy MF, Bassiouny HK, Rashwan A, El-Magdoub AI. *Ambrosia maritima* (Damsissa), a safe and effective molluscicide in the field. *Bull High Inst Public Health*. 1978;8:307–17.
11. Köhler JM, Abahmane L, Wagner J, Albert J, Mayer G. Preparation of metal nanoparticles with varied composition for catalytical applications in microreactors. *Chem Eng Sci*. 2008;63:5048–55.
12. McGillicuddy E, Murray I, Kavanagh S, Morrison L, Fogarty A, Cormican M, et al. Silver nanoparticles in the environment: Sources, detection and ecotoxicology. *Sci Total Environ*. 2017;575:231–46.
13. Rubio CP, Hernández-Ruiz J, Martínez-Subiela S, Tvarijonaviciute A, Ceron JJ. Spectrophotometric assays for total antioxidant capacity (TAC) in dog serum: an update. *BMC Vet Res*. 2016;12:1–7.
14. Lu SC. Regulation of glutathione synthesis. *Mol Aspects Med*. 2009;30:42–59.
15. Docea AO, Calina D, Buga AM, Zlatian O, Paoliello MMB, Mogosanu GD, et al. The effect of silver nanoparticles on antioxidant/pro-oxidant balance in a murine model. *Int J Mol Sci*. 2020;21:1233.
16. Baun A, Hartmann NB, Grieger K, Kusk KO. Ecotoxicity of engineered nanoparticles to aquatic invertebrates: a brief review and recommendations for future toxicity testing. *Ecotoxicology*. 2008;17:387–95.
17. Saitoh Y, Itagaki H. A new breeding system of *Oncomelania hupensis* *nosophora* in the laboratory. *Jpn J Parasitol*. 1980;29:341–50.

18. Michelson EH. Specificity of hemolymph antigens in taxonomic discrimination of medically important snails. *J Parasitol.* 1966;;466–72.
19. Koracevic D, Koracevic G, Djordjevic V, Andrejevic S, Cosic V. Method for the measurement of antioxidant activity in human fluids. *J Clin Pathol.* 2001;54:356–61.
20. Lemaire G, Alvarez-Pachon F-J, Beuneu C, Lepoivre M, Petit J-F. Differential cytostatic effects of NO donors and NO producing cells. *Free Radic Biol Med.* 1999;26:1274–83.
21. Ologhobo AD, Adegede HI, Maduagwu EN. Occurrence of nitrate, nitrite and volatile nitrosamines in certain feedstuffs and animal products. *Nutr Health.* 1996;11:109–14.
22. Oteifa BA, Mousa AH, Abou-El-Hassan AA, Mohamed AM, El-Emam MA. Effect of certain insecticides in the control of the fresh water snails, *Biomphalaria alexandrina* and *Bulinus truncatus*. *Egypt J Bilharz.* 1975;2:221–43.
23. Litchfield JJ, Wilcoxon F. A simplified method of evaluating dose-effect experiments. *J Pharmacol Exp Ther.* 1949;96:99–113.
24. Joseph H. Molluscicidal activity of the plant *Acacia mangium* (Willd.) against the snail *Pomacea canaliculata* (Lam.). *Borneo Akad.* 2016;1:27–33.
25. Navarro E, Baun A, Behra R, Hartmann NB, Filser J, Miao A-J, et al. Environmental behavior and ecotoxicity of engineered nanoparticles to algae, plants, and fungi. *Ecotoxicology.* 2008;17:372–86.
26. Wigginton NS, Titta A de, Piccapietra F, Dobias JAN, Nesatyy VJ, Suter MJ, et al. Binding of silver nanoparticles to bacterial proteins depends on surface modifications and inhibits enzymatic activity. *Environ Sci Technol.* 2010;44:2163–8.
27. Danilczuk M, Lund A, Sadlo J, Yamada H, Michalik J. Conduction electron spin resonance of small silver particles. *Spectrochim Acta A Mol Biomol Spectrosc.* 2006;63:189–91.
28. Kim JS, Kuk E, Yu KN, Kim J-H, Park SJ, Lee HJ, et al. Antimicrobial effects of silver nanoparticles. *Nanomedicine Nanotechnol Biol Med.* 2007;3:95–101.
29. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: a case study on *E. coli* as a model for Gram-negative bacteria. *J Colloid Interface Sci.* 2004;275:177–82.
30. Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ. Metal oxide nanoparticles as bactericidal agents. *Langmuir.* 2002;18:6679–86.
31. Yang XH, Wang JJ, Ge JY, Wang JS, Dong FQ. Controlled synthesis of water soluble silver nanoparticles and the molluscicidal effect. In: *Advanced Materials Research. Trans Tech Publ;* 2012. p. 527–31.

32. Liu J, Hurt RH. Ion release kinetics and particle persistence in aqueous nano-silver colloids. *Environ Sci Technol*. 2010;44:2169–75.
33. Bondarenko OM, Heinlaan M, Sihtmäe M, Ivask A, Kurvet I, Joonas E, et al. Multilaboratory evaluation of 15 bioassays for (eco) toxicity screening and hazard ranking of engineered nanomaterials: FP7 project NANOVALID. *Nanotoxicology*. 2016;10:1229–42.
34. Montassir L, Berrebaan I, Mellouki F, Zkhir F, Boughribil S, Bessi H. Acute toxicity and reprotoxicity of aqueous extract of a Moroccan plant (*Tetraclinis articulata*) on freshwater cladoceran *Daphnia magna*. *J Mater Environ Sci*. 2017;8:770–6.
35. Seremet OC, Olaru OT, Gutu CM, Nitulescu GM, Ilie M, Negres S, et al. Toxicity of plant extracts containing pyrrolizidine alkaloids using alternative invertebrate models. *Mol Med Rep*. 2018;17:7757–63.
36. Ellis L-JA, Kissane S, Hoffman E, Brown JB, Valsami-Jones E, Colbourne J, et al. Multigenerational exposures of *daphnia magna* to pristine and aged silver nanoparticles: epigenetic changes and phenotypical ageing related effects. *Small*. 2020;16:2000301.
37. Hu Y, Chen X, Yang K, Lin D. Distinct toxicity of silver nanoparticles and silver nitrate to *Daphnia magna* in M4 medium and surface water. *Sci Total Environ*. 2018;618:838–46.
38. Reid TJ, Murthy MR, Sicignano A, Tanaka N, Musick WD, Rossmann MG. Structure and heme environment of beef liver catalase at 2.5 Å resolution. *Proc Natl Acad Sci*. 1981;78:4767–71.

Figures

Figure 1

Silver Nano Powder, Type Nano Powder, Diameter 20nm, Format powder, Content 99.9%, SSA 10-20 m²/g, Density 0.25-0.6 g/cm³, Storage Stored at RT.

Figure 2

Toxicity of silver nanoparticles to *Schistosoma mansoni* cercariae (lethal time/minutes).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Graphicalabstract.png](#)