In Vitro Assessment of Anthelmintic Activities of AgO Nanoparticle in Comparison to Closantel Against Liver Fluke Dicrocoelium Dendriticum

Mohsen Arbabi (arbabi4.mohsen@yahoo.com)
Kashan University of Medical Sciences  https://orcid.org/0000-0001-8867-447X

Atefeh Haddad
Kashan University of Medical Sciences

Seyed Mostafa HosseipourMashkani
Institute for Biomedical Materials and Devices, School of Mathematical and Physical Sciences

Hossein Hooshyar
Kashan University of Medical Sciences

Research

Keywords: Dicrocoelium dendriticum, AgO nanoparticle, Scanning Electron Microscopy, in vitro

DOI: https://doi.org/10.21203/rs.3.rs-827258/v1

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

**Background:** Dicrocoeliasis is a rare Food-Born parasitic disease of the grazing herbivores as well as humans, caused by *Dicrocoelium dendriticum* making severe pathological changes of the liver and bile systems, and therapeutic options for treatment are limited. With the appearance of drug resistance in liver flukes, there is a need to focus on alternative approaches to control helminth parasites of veterinary importance. Because of low-performance medications; drug delivery poses a great challenge for better treatment of Dicrocoeliasis. The current study aims to determine the anthelmintic properties of silver oxide nanoparticles (AgO) as a new method in dicrocoeliasis treatment, in vitro assay.

**Methods:** The impacts of various concentrations of AgO nanoparticles (50-200 µg/ml) for 12-24 hours were compared with the Closantel, as the chemical drug. The anthelmintic efficacy was measured by scanning electron microscopy (SEM) technique.

**Results:** SEM images of treated worms by AgO (200 µg/ml) showed severe damage, including complete loss of sensory papillae and destruction of prominent network structures and tegument vesicles. The mortality rates how the anthelmintic properties of AgO were highly relied on time and concentration, as far as increasing the time and concentration cause increasing the mortality rate. According to the MTT assay, the toxicity of AgO, at concentrations, 800 µg/ml is 8.7 %. **Conclusions:** Hence, it could be concluded that AgO NPs performed anthelmintic properties effects. To the best of our knowledge, no previous reports have assessed the effect of AgO NP on liver fluke *D. dendriticum*. Therefore, the present study provides a basis for future research on the control of this common trematode.

**Background**

Dicrocoeliasis is a widespread hepatic bile duct trematode disease, which parasite both humans and a wide range of grazing herbivores and counts as one of the major threats to livestock production in endemic areas [1, 2]. *Dicrocoelium* spp. as well as *Fasciola* spp. designed a group as the food-borne trematodes [3, 4]. The lancet liver fluke, *Dicrocoelium dendriticum* has been distributed throughout Europe, Asia, North Africa, and North America, known as the major cause of the disease. Its parasite is now recognized as Neglected Tropical Diseases, causing major public health problems as well as significant economic impact. Most *D. dendriticum* infections cause no symptoms or only minor ones, hence remain undetected. The clinical infection of dicrocoeliasis is normally resulting in mild symptoms, but heavy infections can lead to serious animal health problems [5, 6]. Dicrocoeliasis causes severe pathological changes of the liver and bile system such as abscesses, granulomas, and fibrosis. Cholangitis with the thickened bile ducts appearing as white spots on cut surfaces of the liver was diagnosed. Chronic disease can develop into cirrhosis [7]. There have been only rare documented cases of re-infection. The parasitizing infectious diseases result in abdominal pain; flatulence, dyspepsia, and watery diarrhea have been reported. Besides, infected animals show a slight increase in their bilirubin (7%) and albumin (3%), not related to the worm burden. However, the clinical symptoms are not pathognomonic in harsh infectious and animal scan causes edemas, anemia, icterus, and a reduction in their production [8].
There are narrow therapeutic choices for the treatment of dicrocoeliasis in animals and drugs need to be used as an unapproved indication. It is troublesome to regulate whether anthelmintic drugs applied at dose rates and routes endorsed for grazing herbivores cause able to eradicate liver fluke in a definitive host as well. The possible hazard of either inefficient levels and the danger of expansion of anthelmintic resistance or leading to toxic levels is accordingly high [9, 10].

Currently, there is no vaccine available for the prevention, and hence chemotherapy is one of the most widely used strategies to control dicrocoeliasis. Nevertheless, due to the emergence of resistance and the cost of treating small ruminants, alternative treatments have been seeking [4]. At present, chemical anthelmintic drugs, including Benzimidazole, pro-benzimidazole families, and Albendazole have been widely used. However, these drugs are not easily available in distant rural areas and also have some serious disadvantages such as the development of drug resistance, adverse drug reactions, residual effects, toxicity problems, and high veterinary costs. Albendazole, which can be used to treat dicrocoeliasis, has been reported to be toxic in camelids [9]. For other (pro) benzimidazoles further studies are required concerning the safety in animals since higher dose rates need to be used against *D. dendriticum* than those used against tapeworms, lungworms, and gastrointestinal nematodes [10]. Therefore, it is vital to design an easily operated and non-invasive compound. In the recent decade, nanoparticles (NPs) due to their defined properties have gained lots of attention and considerable interest which makes them a favorable candidate for anthelmintic application since present antiparasitic drugs have some side effects and their efficacy is not fully proved. Among a wide variety of nanoparticles, Silver nanoparticles (AgNPs) are one of the most vital and fascinating nanomaterials among several metallic nanoparticles that are involved in the biomedical application and created the mass reports in the last years [4, 11]. Notably, dramatic consideration was toward the biomedicine-related appraisal of AgNPs that first invited worldwide consideration as eccentric antimicrobial, antifungal, and antiviral agents. Ag NPs have shown excellent properties against a wide range of microorganisms. One of the most vital and unique applications of AgNPs make them ideal in the field of medicine is using them as antimicrobial agents, as well as for use in nanotoxicology studies [12].

During the past years, AgNPs became one of the most investigated nanostructures that proved to have promised, and interesting characteristics suitable for various unconventional and enhanced biomedical applications [13]. AgNPs are convinced to have legitimate dramatic and countenances capability for the improvement of fiction antimicrobial agents, drug-delivery formulations, identification and diagnosis platforms, biomaterial and medical apparatus blankets, and performance-enhanced therapeutic alternatives [14]. However, their mechanism of action is not completely clarified. Several mechanisms have been assumed for the effect of silver NPs on the cell. First of all, direct contact with microorganisms, the ability to penetrate to cell walls, changing the structure of cell membranes, and finally resulting in cell death. Second of all, by binding to the proteins, in the cell membrane, which is involved in the generation and transportation of APP [15]. Thus, the current study aimed to investigate the in vitro anthelmintic activities of AgONPs, against adult *D. dendriticum* in comparison with the chemical drug, Closantel, and the negative control, RPMI culture media. Surface alterations to the tegument were evaluated by SEM technique and were applied to investigate the concentration effect of AgONPs and with
the impact of different incubation times on the death rate of *D. dendriticum* in comparison with Closantel. However, to the best of the author's knowledge, this is the first report on the impact of AgO on the structure and motility of adult *D. dendriticum*.

**Methods**

**Materials and physical measurements**

All synthesized nanoparticles used in this method were of analytical grade and used as received without any further purification. X-ray diffraction (XRD) patterns were recorded by a Philips-X’PertPro, X-ray Diffractometer using Ni-filtered Cu Ka radiation at scan range of 10 °<θ<80. The size and shape of metal nanoparticles are measured by scanning electron microscopy (SEM). SEM images were obtained on LEO-1455VP equipped with energy-dispersive X-ray spectroscopy. Spectroscopy analysis (UV-Vis) was carried out using Shimadzu UV-Vis scanning UV-Vis diffuse reflectance spectrometer. The energy dispersive spectrometry (EDS) analysis was studied by XL30, Philips microscope.

**Synthesis of AgO nanoparticle**

To synthesize AgONPs, 1 g of silver nitrate was dissolved in 20 mL of deionized water under ultrasonic irradiation (180 W) and stirring to make a clear solution. Afterward, 1.5 g of potassium persulfate (K₂S₂O₈) as the powerful oxidant was added to the above solution under ultrasonic irradiation (180 W) and stirring for 15 min. Finally, the gray precipitate was filtered and washed three times with distilled water and then was dried at 60°C for 24 hours.

**Collection of *D. dendriticum***

Adult fresh *D. dendriticum* were collected from the liver of slaughtered goats and sheep from the Kashan slaughterhouse. The samples after transported to the laboratory were washed three times with PBS buffer (pH:7-7.3) for future processing. Finally, they were immediately transferred into the 24 well plates containing RPMI1640 (50 IU/ml of penicillin, 50 IU/ml of streptomycin, 50 % V/V of Fetal bovine serum (FBS), and 2% of Sheep red blood cells for different treatment. Only worms with normal tegument and exhibit motility by visual inspection were selected.

**MTT viability assay**

The MTT assay was used to measure the viability of the Hela cells to find the optimum concentrations of AgO. Some 10⁵Hela cells per well were placed in 96-well plates along with introducing different concentrations of AgO to each well and keeping at 37°C, 5% CO₂ for 24 h. Afterward, the MTT solution (20 µl, 5 mg/ml in PBS) was added to each well and further incubated for 3 h. After 3 hours, the supernatant of each well was removed and 100 µl of DMSO was added to each well. After 15 min incubation with DMSO, the ELISA plate reader was used for reading the absorbance of each well at 570 nm [16]. The percentage of cell viability was calculated based on Eq. 1 [17].
Cell Viability (%) = 100 * OD sample/OD control

**In vitro assays**

The investigation was carried out in four groups. Every treatment considered of different concentrations of experimental groups NPs: 50, 100, 150, and 200 µg/ml), positive control treatment (Closantel: 50, 100, 150, and 200 µg/ml) and negative control treatment (only RPMI1640 media culture). For this purpose, under sterile conditions in a laminar flow cabinet, adult liver flukes *D. dendriticum* were transferred into the 24 well, each well contains 1ml of RPMI1640 (50IU/ml of penicillin, 50IU/ml of streptomycin, 50% V/V of FBS, and 2% of sheep red blood cells). Afterward, 1ml of AgONPs were added individually to each well and incubated for 12, 18, and 24h at 37°C in an atmosphere of 5% CO₂. The number of live adult *D.dendriticum* was checked during these times.

**Motility examination**

The motility time (12, 18, and 24 h) of worms after incubation in different concentrations of the treatments and control groups was calculated and the viability of experiments was measured based on the motility criteria (the whole body moving high, parts of the body moving, less movement of the whole body and complete loss of motility). The trematodes revealed no movement visually were examined by a light microscope for movement conformation. The motility assay in control groups was noted, according to the absence of the experimental compounds. All worms in each experiment were observed individually at an hour interval according to the motility criteria established.

**Scanning electron microscopy (SEM) sample preparation**

For the determination of the ultrastructural alteration in the tegumental of worms in the test and control groups, we used electron microscopy (SEM). In the first step, the treated and control flukes were fixed with sodium cacodylate buffer (pH: 7.4, 0.2 M) and glutaraldehyde in phosphate buffer (2.5 % v/v) for 4 h at 4°C, and then the parasites were washed 3 times in phosphate buffer (pH: 7.4). In the second step, they were dehydrated in ascending ethanol concentrations (70%, 80%, 95%, and 100%) for 30 min, final dehydration was achieved in hexamethyldisilazane and consequently dried in the vacuum oven. Finally, mounted on stubs, sputter-coated with gold, and they were photographed by SEM (ZEISS-960A, Germany) at the central laboratory of the Institute for Color Science and Technology, Tehran, Iran.

**Results**

**Structural study**

Crystal structure and phase purity of the as-synthesized AgONPs were measured by X-ray powder diffraction (XRD). Based on the XRD pattern of Silver Oxide NPs (Fig. 1), the observed diffraction peaks can be indexed to the pure Monoclinic phase of AgO with space group P21/c (JCPDS no. 80-1269). The crystallite diameter (Dc) of AgONPs based on the Scherrer equation (Dc= Kλ/ βcosθ) was calculated to be 19.59 nm. Where β is the breadth of the observed diffraction line at its half intensity maximum, K is the
so-called shape factor, which usually takes a value of about 0.9, and $\lambda$ is the wavelength of the X-ray source used in XRD. Further prove the composition of AgO was performed by energy dispersive spectroscopy (EDS), as shown in Fig. 2 a. EDS spectrum reveals that NPs composed of only Ag and O atoms. Fig. 2 b demonstrates the SEM images of Silver Oxide NPs. It showed AgO mainly consists of rice shape NPs with an average size of 20-40 nm. Besides, Furrier transform infrared spectroscopy (FT-IR) was also carried out to check the presence of certain functional groups in AgONPs, as depicted in Fig. 3 a. FT-IR spectrum shows three absorption bands at 3182, 1059, 705 cm$^{-1}$ which can be attributed to the stretching and bending vibrations of H$_2$O molecules (3182 and 1059 cm$^{-1}$) and the Ag-O bond (705 cm$^{-1}$). The optical property of as-synthesized AgONPs was determined using UV-vis spectroscopy. Fig. 3 b depicts $(\alpha h \nu)^{1/2}$ vs h curve of AgONPs which was calculated based on the Wood and Tauc equation [2].

\[
\alpha h^n = (h \nu - E_g)^n
\]

where $\alpha$ is the absorbance, h the Planck constant, $\nu$ the photon frequency, $E_g$ the energy gap, and n the pure numbers associated with the different types of electronic transitions. For n=1/2, 2, 3/2, and 3, the transitions are directly allowed, indirectly allowed, directly forbidden, and indirectly forbidden, respectively. Each energy gap was determined by the extrapolation of each linear portion of the curves to $\alpha = 0$. Hence, the energy gap of AgONPs was calculated to be 2.9 eV.

**Anthelmintic treatment study**

**In vitro toxicity assay**

Toxicity values (TC50) of HeLa cells were measured after 24 h incubation and were compared with positive and negative control groups. The toxicity results of AgO were shown in Table 1 and Fig. 4. The toxicities of AgO at 100 µg/ml were 8.7 %. Results indicated the toxicity of AgO NPs has low.

**Worm motility assays**

The number of viable adult worms was measured by the eosin staining method after 12, 18, and 24 h incubation with AgO, and Closantel respectively. The results clearly showed that after 12 h incubation at concentration 150µg/ml of AgONPs as well as the highest concentration to 200 µg/ml all worms were dead. Furthermore, after 12 h incubation at 200µg/ml of Closantel, all adult *D. dendriticum* died. Results indicated that less motile with the increasing concentration of the AgONPs. On the other hand, the decrease in the motility rate of flukes treated with experimental drugs was both time and concentration-dependent (Table 2).

**Scanning electron microscopy (SEM) of adult *D. dendriticum***

The tegumental topographical changes of *D. dendriticum* liver flukes were investigated by the SEM technique to assess the effects of AgO on their surface structures. The control worms seem normal with
unchanged tegument around suckers and their oral and ventral suckers are round and smooth. Besides, sensory papillae at the edges and inside the oral sucker, tegumental ridges network, and vesicles look unaltered. Besides, ridge walls and valley floors cover densely by tegumental vesicles in the entire body, which seem to be like spherical structures (Fig. 5, a-d). SEM images of treated worms with AgO NPs (100 µg/ml) demonstrated that their tegumental region endured a variety of changes, including appearing severe swelling, swollen and blister (Fig. 6a), destroying sensory papillae (Fig. 6b), and also destroying the network structure and tegument vesicles (Fig. 6, c, d). SEM images of treated *D. dendriticum* with Closantel (200 µg/ml) are shown in Fig. 7a-f. Based on Fig. 7a, swelling, erosions, and blebs appeared on the surface tegumental. Besides, cirri were damaged and lost their natural appearance (Fig. 7b). Also, sensory papillae were disappeared completely and oral and ventral suckers are flaky completely insofar as a little recognizable structure was remained (Fig. 7c-f).

**Discussion**

Dicrocoeliasis, caused by *Dicrocoelium dendriticum*, is a common disease among ruminates that has important economic and veterinary aspects [8]. Although anti-parasitic medicine, chemicals are readily available, they have irreversible side effects. Among the different groups of metallic nanoparticles, silver nanoparticles (Ag-NPs) have become one of the common in-demand nanoparticles in debt to their exponential number of operations in various districts. The increased use of Ag-NPs-enhanced products may result in an increased level of toxicity affecting living organisms such as parasites [17, 19, 20]. Given the widespread use of AgNPs, risk assessment of these nanoparticles is of great importance. Numerous studies have demonstrated the potency of AgNPs to induce deleterious biological and cellular effects [21]. These nanoparticles adhere to the cell walls and membranes of microorganisms and may reach the cell interior. They injure the cellular organelles, engender the production of reactive oxygen species, and alter the mechanisms of signal transduction [22]. Several studies report utilisations in which good results have been achieved using silver nanoparticles for the control of pathogenic microorganisms in the fields of public health and medicine [14, 23]. Despite the widespread use of silver nanoparticles, few studies have been performed on AgNPs against platyhelminth parasitic infections [18, 24–26]. Besides, many investigations have been performed on the anti-parasitic impact of the AgNPs on the *Gigantocotyle explanatum, Haemonchus contortus, Ancylostoma caninum*, and *Fasciola hepatica* [26–29]. It has been suggested that metal oxide NPs such as AgO have a high attitude to create reactive oxygen species (ROS) and free radicals, which are responsible for causing oxidative stress and apoptosis leading to cell death, which ends up with acceptable antibacterial, antifungal, antioxidants and anti-parasite [30, 31]. Indeed, the enormous creation of ROS in cells by direct interaction with particles is at present accepted as one of the major mechanisms of cellular toxicity of nanoparticles [1, 32–34]. ROS have many signaling and information functions; however, excessive ROS can collapse the antioxidant defense system, leading to the damage of DNAs, lipids, and proteins [34, 35]. Ag NPs with larger surface areas provide better contact with microorganisms. Since Ag NPs are smaller than microorganisms, they easily diffuse into cells and rupture the cell wall and pathogens. It has also been shown that smaller nanoparticles are more toxic than bigger ones. The toxicity of Ag NPs is dependent on the concentration, pH of the medium, and
exposure time to pathogens [36]. Also, their efficacy is due not only to their nanoscale size but also to their large ratio of surface area to volume. They can increase the permeability of cell membranes, produce reactive oxygen species, and interrupt the replication of deoxyribonucleic acid by releasing silver ions [37]. Also, antioxidant enzymes have been recognized as important modulators in AgNP induced oxidative stress. Two of them, catalase (CAT) and superoxide dismutase (SOD), are prominent for maintaining the level of ROS in organisms and are used as bio-indicators of increased ROS production [17]. Previous research demonstrated that AgNPs induce oxidative stress by altering the activity of both enzymes in vivo and in vitro assays [38]. It is currently accepted that the alteration of the enzyme activity might be due to either regulation of genes or to direct surface interaction of the enzymes with AgNPs [39]. The molecular mechanisms of the interaction between enzymes and nanoparticles were also explored in some in vitro studies [40–42]. The organisms treated by Ag NPs are under oxidative stress and induced autophagic cell death and mitochondrial damage via reactive oxygen species generation along with the inhibition of the major antioxidant enzyme, SOD [43, 44].

In the current study, the effect of AgO NPs on adult *D. dendriticum* was investigated using in vitro model. Anti-parasitic results of AgO NPs demonstrated that 100 µg/ml of AgO at 24 h shows a high destruction effect on the tegument surface of *D. dendriticum*. Future research is needed to realize the mechanism of action of AgNPs in the tegument of this liver fluke as well as to detect internal injure to the parasitic ultrastructure and molecular changes dominant to parasite death.

**Conclusion**

In summary, scanning electron microscopy (SEM) was applied to the anthelmintic efficiency of AgO NPs. Besides, the impacts of various concentrations and reaction time on the anthelmintic efficiency of AgO NPs were also investigated. Also, the efficiency, mortality rate, which is based on the number of live and dead adult *D. dendriticum*, of NPs was investigated after 12, 18, and 24 h expose time. SEM results demonstrated that NPs showed dose-dependent anthelmintic efficiency. Nonetheless, additional research is necessary to evaluate the in vivo the efficacy of this treatment as well as its toxicity on a definitive host. Oxidative stress is accepted as a key mechanism of AgNPs toxicity in living organisms. Therefore, mechanistic studies related to the impact of AgNPs on the structure and function of antioxidant enzymes at the molecular level are essential for a comprehensive evaluation of their toxicity.

**Declarations**

**Acknowledgments**

The authors are grateful to Deputy of Research, Kashan University of Medical Sciences, Kashan, Iran, for its support.

**Authors’ contributions**
Designing the study: MA. Collecting data and statistical analysis: MA and AH. Literature review: MA, AH, SMHM, HH. Data interpretation: MA, SMHM. Drafting the manuscript: MA. Critical revision of manuscript: MA, AH. All authors read and approved the final manuscript.

Funding

The present study was supported by the Deputy of Research, Kashan University of Medical Sciences, Kashan, Iran, through grant no. 9740.

Availability of data and materials

The datasets used analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This project was found to be in accordance with the ethical principles and the national norms and standard for conducting Medical Research in Iran. The study protocol was approved by the Ethics Committee of Kashan University of Medical Sciences, Iran, (Approval ID is IR.KAUMS.MEDNT.REC.2018.23).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Authors' information (optional)

1Department of Medical Parasitology and Mycology, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran. 2Department of Medical Parasitology and Mycology, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran. 3Institute for Biomedical Materials and Devices, School of Mathematical and Physical Sciences, Faculty of Science, University of Technology Sydney, NSW 2007, Australia. 4Department of Medical Parasitology and Mycology, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran.

References

1. Arias M, Lomba C, Dacal V, Vázquez L, Pedreira J, Francisco I, et al. Prevalence of mixed trematode infections in an abattoir receiving cattle from northern Portugal and north-west Spain. Vet Rec. 2011;168(15):408.
2. Scala A, Tamponi C, Dessì G, Sedda G, Sanna G, Carta S, et al. Dicrocoeliosis in extensive sheep farms: a survey. Parasites vectors. 2019;12(1):342.

3. Keiser J, Utzinger J. Food-Borne trematodiases. Clin Microbiol Rev. 2009;22:466–83.

4. Fairweather I, Brennan G, Hanna R, Robinson M, Skuce P. Drug resistance in liver flukes. International Journal for Parasitology: Drugs Drug Resistance. 2020;12:39–59.

5. Arbabi M, Nezami E, Hooshyar H, Delavari M. Epidemiology and economic loss of fasciolosis and dicrocoeliosis in Arak. Iran Veterinary world. 2018;11(12):1648–55.

6. Majidi-Rad M, Meshgi B, Bokaie S. The prevalence and intensity rate of Dicrocoelium dendriticum infection in ruminants of 3 provinces in coastal regions of the Caspian Sea. Iranian Journal of Veterinary Medicine. 2018;12(1):27–33.

7. Hilbe M, Robert N, Pospischil A, Gerspach C. Pulmonary arterial lesions in New World camelids in association with Dicrocoelium dendriticum and Fasciola hepatica infection. Vet Pathol. 2015;52(6):1202–9.

8. Manga-González MY, Ferreras M, Campo R, González-Lanza C, Perez V, García-Marín JF. Hepatic marker enzymes, biochemical parameters and pathological effects in lambs experimentally infected with Dicrocoelium dendriticum (Digenea). Parasitol Res. 2004;93(5):344–55.

9. Gruntman A, Nolen-Walston R, Parry N, Wilborn R, Maxwell H. Presumptive albendazole toxicosis in 12 alpacas. Journal of Veterinary Internal Medicine. 2009;23(4):945–9.

10. Dadak AM, Wieser C, Joachim A, Franz S. Efficacy and safety of oral praziquantel against Dicrocoelium dendriticum in llamas. Vet Parasitol. 2013;197:122–5.

11. Abaza SM. Applications of nanomedicine in parasitic diseases. Journal of the Egyptian Parasitologists United. 2016;9(1):1–6.

12. Zhang XF, Liu ZG, Shen W, Gurunathan S. Silver Nanoparticles: Synthesis, Characterization, Properties, Applications, and Therapeutic Approaches. International Journal of Molecular Sciencesi. 2016;13(9):1534. 17(.

13. Soliman H, Elsayed HA, Dyaa A. Antimicrobial activity of silver nanoparticles biosynthesized by Rhodotorula sp. strain ATL72. Egyptian Journal of Basic Applied Sciences. 2018;5:228–33.

14. Burdus AC, Gherasim O, Grumezescu AM, Mogoanta L, Ficai A, Andronescu E. Biomedical Applications of Silver Nanoparticles: An Up-to-Date Overview. Nanomaterials. 2018;8:8,681.

15. Klueh U, Wagner V, Kelly S, Johnson A, Bryers J. Efficacy of silvercoated fabric to prevent bacterial colonization and subsequent devicebased biofilm formation. Journal of Biomedical Materials Research: An Official Journal of The Society for Biomaterials. 2000;53(6):621–31.

16. Fisher GA, Sikic BI. Clinical studies with modulators of multidrug resistance. Hematology/ Oncology Clinics. 1995;9(2):363–82.

17. Akter M, Sikder T, Rahman M, K.M.A.Ullah A, Hossain KFB, Banik S, Hosokawa T, Saito T, Kurasaki M. A systematic review on silver nanoparticles-induced cytotoxicity: Physicochemical properties and perspectives. J Adv Res. 2018;9:1–16.
18. Lashkarizadeh MR, Asgaripour K, Dezaki ES, Harandi MF. Comparison of scolicidal effects of amphotericin B, silver nanoparticles, and Foeniculum vulgare Mill on hydatid cysts protoscoleces. Iranian Journal of Parasitology. 2015;10(2):206–12.

19. Gupta RK, Kumar V, Gundampati RK, Malviya M, Hasan SH, Jagannadham MV, et al. Biosynthesis of silver nanoparticles from the novel strain of Streptomyces Sp. BHUMBU-80 with highly efficient electroanalytical detection of hydrogen peroxide and antibacterial activity. Journal of Environmental Chemical Engineering. 2017;5:5624–35.

20. Loo YY, Rukayadil Y, Nor-Khaizura MAR, Kuan CH, Chieng BW, Nishibuchi M, et al. In vitro antimicrobial activity of green synthesized silver nanoparticles against selected gram-negative foodborne pathogens. Front Microbiology. 2018;9:1555.

21. Cameron SJ, Hosseinian F, Willmore WG. A Current Overview of the Biological and Cellular Effects of Nanosilver. Int J Mol Sci. 2018;19:1–40.

22. Dakal TC, Kumar A, Majumdar RS, Yadav V. Mechanistic basis of antimicrobial actions of silver nanoparticles. Front Microbiology. 2016;16:1831.

23. Mishra S, Singh HB. Biosynthesized silver nanoparticles as a nanoweapon against phytopathogens: exploring their scope and potential in agriculture. Appl Microbiol Biotechnol. 2015;99:1097–107.

24. Singh SK, Goswami K, Sharma RD, Reddy MV, Dash D. Novel microfilaricidal activity of nanosilver. Int J Nanomed. 2012;7:1023–30.

25. Gherbawy YA, Shalaby IM, El-sadek MSA, Elhariry HM, Banaja AA. The anti-fasciolasis properties of silver nanoparticles produced by Trichoderma harzianum and their improvement of the anti-fasciolasis drug triclabendazole. Int J Mol Sci. 2013;14(11):2188–98.

26. Preet S, Tomar RS. Anthelmintic effect of biofabricated silver nanoparticles using Ziziphus jujuba leaf extract on nutritional status of Haemonchus contortus. Small Ruminant Research. 2017;154:45–51.

27. Aziz A, Raju GS, Das A, Ahmed J, Moghal MMR. Evaluation of in vitro anthelmintic activity, total phenolic content and cytotoxic activity of Crinum latifolium L. (Family: Amaryllidaceae). Advanced Pharmaceutical Bulletin. 2014;4(1):15–9.

28. Barbosa ACMS, Silva LPC, Ferraz CM, Tobias FL, de Araújo JV, Loureiro B, et al. Nematicidal activity of silver nanoparticles from the fungus Duddingtonia flagrans. Int J Nanomed. 2019;14:2341–8.

29. Rehman A, Ullah R, Uddin I, Zia I, Rehman L, Abidi S. In vitro anthelmintic effect of biologically synthesized silver nanoparticles on liver amphistome, Gigantocotyle explanatum. Exp Parasitol. 2019;198:95–104.

30. Unfried K, Albrecht C, Klotz L-O, Von Mikecz A, Grether-Beck S, Schins RP. (2007). Cellular responses to nanoparticles: target structures and mechanisms. Nanotoxicology, 1(1):52–71.

31. Allahverdiyev AM, Abamor ES, Bagirova M, Ustundag CB, Kaya C, Kaya F, et al. Antileishmanial effect of silver nanoparticles and their enhanced antiparasitic activity under ultraviolet light. Int J Nanomed. 2011;6:2705–14.
32. von Moos N, Slaveykova VI. Oxidative stress induced by inorganic nanoparticles in bacteria and aquatic microalgae—state of the art and knowledge gaps. Nanotoxicology. 2014;8(6):605–30.

33. Marchioni M, Jouneau P-H, Chevallet M, Michaud-Soret I, Deniaud A. Silver nanoparticle fate in mammals: Bridging in vitro and in vivo studies. Coord Chem Rev. 2018;364:118–36.

34. Duran N, Duran M, de Jesus MB, Seabra AB, Favaro WJ, Nakazato G. Silver nanoparticles: A new view on mechanistic aspects on antimicrobial activity. Nanomedicine. 2016;12(3):789–99.

35. He D, Dorantes-Aranda JJ, Waite TD. Silver Nanoparticle - Algae Interactions: Oxidative Dissolution, Reactive Oxygen Species Generation and Synergistic Toxic Effects. Environmental Science Technology. 2012;46(16):8731–8.

36. Siddiqi KS, Husen A, Rao RAK. A review on biosynthesis of silver nanoparticles and their biocidal properties. Journal of Nanobiotechnology. 2018;16:14.

37. Yin IX, Zhang J, Zhao IS, Mei ML, Li Q, Chu CH. The Antibacterial Mechanism of Silver Nanoparticles and Its Application in Dentistry. Int J Nanomed. 2020;15:2555–62.

38. Ahamed M, Posgai R, Gorey TJ, Nielsen M, Hussain SM, Rowe JJ. Silver nanoparticles induced heat shock protein 70, oxidative stress and apoptosis in Drosophila melanogaster. Toxicol Appl Pharmacol. 2010;242(3):263–9.

39. Pudlarz AM, Ranoszek-Soliwoda K, Czechowska E, Tomaszewska E, Celichowski G, Grobelny J, et al. A Study of the Activity of Recombinant Mn-Superoxide Dismutase in the Presence of Gold and Silver Nanoparticles. Appl Biochem Biotechnol. 2019;187(4):1551–68.

40. Zhang HM, Cao J, Tang BP, Wang YQ. Effect of TiO(2) nanoparticles on the structure and activity of catalase. Chem Biol Interact. 2014;219:168–74.

41. Wang Y, Zhang H. Comprehensive studies on the nature of interaction between catalase and SiO2 nanoparticle. Mater Res Bull. 2014;60:51–6.

42. Fang W, Chi Z, Li W, Zhang X, Zhang Q. Comparative study on the toxic mechanisms of medical nanosilver and silver ions on the antioxidant system of erythrocytes, from the aspects of antioxidant enzyme activities and molecular interaction mechanisms. Journal of Nanobiotechnology. 2019;17(1):66.

43. Yu KN, Yoon TJ, Minai-Tehrani A, Kim JE, Park SJ, Jeong MS, et al. Zinc oxide nanoparticle induced autophagic cell death and mitochondrial damage via reactive oxygen species generation. Toxicol In Vitro. 2013;27(4):1187–95.

44. Saini P, Saha SK, Roy P, Chowdhury P, Babu SPS. Evidence of reactive oxygen species (ROS) mediated apoptosis in Setaria cervi induced by green silver nanoparticles from Acacia auriculiformis at a very low dose. Experimental parasitology. 2016;160:39–48.

Tables

Table 1. Toxic values (TC50) of Hela cell exposed to the AgO nanoparticles
| Concentration (µg/ml) | Viability (%) |
|----------------------|---------------|
| 50                   | 100           |
| 100                  | 99.3          |
| 150                  | 80.2          |
| 200                  | 79.3          |

**Table 2.** Comparison Lethal concentration (LC) values of AgO with control group (Closantel) during 12, 18, and 24 h.

| Groups              | LC (Hour) | LC10 | LC25 | LC50 | LC75 | LC90 |
|---------------------|-----------|------|------|------|------|------|
| AgO nanoparticle    | 12        | 87.9 | 95.03| 102.9| 110.9| 120.1|
|                     | 18        | 39.7 | 60.6 | 83.8 | 101.2| 115.1|
|                     | 24        | 39.1 | 45.8 | 52.5 | 59   | 62.5 |
| Closantel           | 12        | 89.5 | 106.3| 125  | 143.6| 160.4|
|                     | 18        | 89.5 | 106.3| 125  | 143.6| 160.4|
|                     | 24        | 87.8 | 95   | 102.9| 110.9| 118  |

Comparison between groups P<0.001

**Figures**
Figure 1

XRD pattern of Silver Oxide nanoparticle.
Figure 2

(a) EDS spectrum (b) SEM images of Silver Oxide nanoparticle nanoparticles with diameters of 20 nm
Figure 3

FT-IR pattern (b) UV-Vis pattern of Silver Oxide nanoparticle.

Figure 4

TC50 values of Hela cell after 24 h incubation with AgO nanoparticle.
Figure 5

SEM images of adult D. dendriticum (Control) worms incubated in RPMI showing: (a, b) round and smooth oral sucker (OS) and ventral sucker (VS), larger than OS, (c) normal and intact tegumental enfolding's around sucker, sensory papillae at the edges and inside OS, (d) tegumental ridges and vesicles covering the valley floors.
Figure 6

SEM showing the effect of 100 µg/ml AgO NPs on the tegumental surface of adult D. dendriticum: (a) swollen and blister on the surface of tegument, (b) loss of sensory papillae and severe tegumental damage, (c,d) complete destruction of prominent network structure and tegument vesicles.
Figure 7

SEM showing the effect of 200 µg/ml Closantel the tegumental surface of adult D. dendriticum: (a) swelling on the surface of tegument, (b) loss of natural morphology of Cirrus and sever damage (c-e) complete destruction of prominent network structure and tegument vesicles, (f) complete loss of sensory papillae.