Received: 30 September 2023 | Revised: 19 January 2024 | Accepted: 22 February 2024

DOI: 10.1002/jemt.24542

RESEARCH ARTICLE

MICROSCOPY RESEARCH & TECHNIQUE WILEY

Light microscopical and parasitological analyses revealed the beneficial effects of silver nanoparticles and various myrrh extracts against *Trichinella spiralis* infection in mice

Nahed A. Elossily[1] | Salwa M. Abd-ELrahman[2] | Abeer A. Khedr[3] | Ahmed K. Dyab[1] | Abeer E. Mahmoud[1] | Shaymaa M. Mohamed[4] | Ahmed M. Abd Elrahman[5] | Fahd M. Alsharif[6] | Reem M. Alsaadawy[7] | Ramy K. A. Sayed[8] | Mervat M. Khalifa[1]

[1]Department of Medical Parasitology, Faculty of Medicine, Assiut University, Assiut, Egypt

[2]Department of Parasitology, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

[3]Department of Parasitology, Faculty of Veterinary Medicine, New Valley University, New Valley, Egypt

[4]Department of Pharmacognosy, Faculty of Pharmacy, Assiut University, Assiut, Egypt

[5]Department of Chemistry, Faculty of Science, Assiut University, Assiut, Egypt

[6]Department of Pharmaceutics and Ind. Pharmacy, College of Pharmacy, Al-Azhar University, Assiut, Egypt

[7]Department of Zoonoses, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

[8]Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt

Correspondence
Ramy K. A. Sayed, Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Sohag University, Sohag 82524, Egypt.
Email: ramy.kamal@vet.sohag.edu.eg

Review Editor: Mingying Yang

Abstract

Trichinella spiralis infection is a food-borne zoonotic disease caused by nematodes that dwell in the tissues, presenting a significant public health concern. This study aimed to evaluate the effectiveness of different treatments including silver nanoparticles (AgNPs), myrrh biosynthesized AgNPs "AgNPs synthesized using plant-based green technologies", myrrh extract, and myrrh essential oil, as alternative treatments against *T. spiralis* infection. Parasitological, histopathological, and cytotoxicity assessments were conducted to investigate the effects of various concentrations of these treatments in reducing the populations of adult worms and larvae during both the intestinal and muscular phases of *T. spiralis*-infected mice. The results showed that the highest antihelminthic efficacy against the intestinal phase of *T. spiralis* was achieved by myrrh extract (86.66%), followed closely by AgNPs (84.96%) and myrrh AgNPs (82.51%) at higher concentrations (800 mg/kg for myrrh extract, 40 µg/mL for AgNPs, and 40 µg/mL for myrrh AgNPs). While the group treated with myrrh essential oil showed the lowest percentage of adult reduction (78.14%). However, all treatments demonstrated comparable effects in reducing the larvae population in the muscle phase. Histopathological examination of the tissues revealed compelling evidence of the effectiveness of AgNPs, particularly when prepared with myrrh. Additionally, a comprehensive assessment of the cytotoxicity of AgNPs indicated low toxicity levels. This study supports that AgNPs synthesized using plant-based green technologies hold therapeutic potential for the treatment of *T. spiralis* infection.

These findings present a promising avenue for the development of novel antiparasitic drugs that are both effective and safe.

Research Highlights

- Myrrh extract has the highest antihelminthic efficacy against the intestinal phase of *T. spiralis*.
- Histopathological examination of the tissues revealed compelling evidence of the effectiveness of AgNPs, particularly when prepared with myrrh.
- During intestinal phase of *T. spiralis*, varying levels of nanoparticle precipitation were detected in the liver, brain, lung, and intestine. During the muscular phase, the highest amount of AgNPs precipitation was detected in the liver, followed by the brain, and lung.

KEYWORDS

AgNPs, cytotoxicity, skeletal muscle, *T. spiralis*, volatile oil of myrrh

1 | INTRODUCTION

Trichinella spiralis is a tissue-dwelling cylindrical worm that causes a food-borne zoonotic disease termed Trichinellosis (Lopez-Cauce et al., 2022). Many host species are vulnerable to *Trichinella* sp. infection, with humans being the most susceptible (Gomez-Morales et al., 2022). The majority of human infections are caused by eating meat (most commonly pig meat), either raw or improperly cooked, that contains infectious larvae (Gabriël et al., 2023). The World Health Organization (WHO) and the Food and Agriculture Organization (FAO) have identified *T. spiralis* infection as the sixth most serious food-borne parasite of global concern (Lupşe et al., 2023).

T. spiralis undergoes three distinct clinical phases within the host: enteral, migratory, and muscular (Abou Rayia et al., 2022). The life cycle commences with ingesting larvae encysted in muscle tissue by an infected host, typically a carnivorous mammal, pig, or wild game animal. Following ingestion, the larvae are released in the stomach due to digestive enzymes, where they mature into adult worms in the small intestine. After mating, females release larvae that traverse the intestinal wall, enter the bloodstream, and reach various muscles. In the muscular phase, larvae penetrate skeletal muscle fibers establishing a specialized environment by transforming infected myocytes into nursing cells (Duignan, 2023). This shelter, comprising host-origin components, effectively shields the larvae from the host's immune response (Guo et al., 2020). Consequently, patients may undergo myositis and muscular dysfunction, induced by larval growth in skeletal muscles, leading to severe and prolonged pain (Abou Rayia et al., 2022; Ahmad et al., 2023).

The therapeutic options for *T. spiralis* infection are notably restricted, and the existing drugs employed against *Trichinella*, such as mebendazole and albendazole, possess limited water solubility. Consequently, their absorption from the intestinal lumen is diminished, necessitating high doses that lead to a multitude of adverse effects and contribute to the development of drug resistance. Furthermore, these drugs exhibit limited efficacy in combating the encapsulated larvae (Allam et al., 2021).

Hence, the development of new anti-*Trichinella* drugs that are both safe and efficacious against the muscular phase of the infection is imperative. In the realm of medical applications, silver stands out as the most extensively employed engineered nanomaterial. Silver nanoparticles (AgNPs), in particular, exhibit potent antimicrobial properties owing to their expansive surface area, facilitating enhanced interaction with microorganisms when compared with other salts (Dara et al., 2020; Salleh et al., 2020). Consequently, AgNPs have exhibited a growing ability to penetrate various human body tissues, cells, and biological molecules (Mehwish et al., 2021). Notably, AgNPs have demonstrated promising therapeutic potential in parasitic diseases that specifically target certain cells or organs (Ayipo et al., 2022). For instance, in vitro studies have revealed significant interactions between different concentrations of AgNPs and *T. spiralis* adults and larvae, leading to cuticle degeneration, which could potentially impede their in vivo binding ability to host cells (Khalifa et al., 2023).

Nonetheless, evidence suggests that AgNPs can have adverse effects on human health and the environment (Tortella et al., 2020). Consequently, extensive research has been conducted to mitigate the toxic effects of AgNPs while enhancing their potential (Bruna et al., 2021). Among the various production processes, biological or green synthesis of AgNPs has garnered significant attention due to its nontoxic, environmentally friendly nature (Chouhan & Guleria, 2020). Therefore, the utilization of plant extracts for the mediated synthesis (green synthesis) of nanoparticles is preferred, given that plants possess the inherent ability to reduce silver ions into AgNPs (Bergal et al., 2022). Myrrh is a natural remedy, being an oleo-gum resin from *Commiphora* species, which has been employed as a reducing and capping agent for the synthesis of AgNPs demonstrating a high degree of effectiveness against both the intestinal and muscular phases of *T. spiralis* (Abd-Elrahman et al., 2020).

The primary objective of this experimental study was to investigate the effectiveness of AgNPs as a potential therapeutic agent against both the intestinal and muscular phases of *T. spiralis* infection by assessing their impact on the host. Furthermore, the study aimed to explore the host–parasite interaction under the treatment protocols, which involved evaluating parasitological and histopathological parameters, as well as assessing the cytotoxicity of the nanoparticle combination.

2 | MATERIALS AND METHODS

The national and international ethical guidelines were followed in the present study. The work was approved (Approval number: 17300257) by the research ethical committee of the Faculty of Veterinary Medicine, Assiut University, under ARRIVE guidelines. Mice were euthanized by cervical dislocation under anesthesia.

2.1 | Experimental animals and *T. spiralis* strain

The current study was carried out on 120 BALB/c mice, aged 7–8 weeks and weighing 20–25 g. The animals were purchased and housed at the animal house of Assiut University (Assiut, Egypt). Throughout the whole period of study, the mice were kept under specific pathogen-free conditions with free access to tap water and a standard diet that contained 7% simple sugars, 3% fat, 50% polysaccharide, 15% protein, and energy 3.5 kcal/g (Longo et al., 2014).

T. spiralis strain was isolated from a naturally infected pig slaughtered at El-Bassatine Abattoir, Cairo, Egypt. The larvae were isolated from the skeletal muscle and then maintained in vivo in BALB/c mice (Abd-Elrahman et al., 2021).

2.2 | Experimental design

This study involved a total of 120 mice, divided into two main groups; to assess the effectiveness of various treatments. Each main group, comprising 60 mice, was subdivided into six subgroups, each receiving distinct treatments. For each treatment group, two concentrations were utilized, except for the infected untreated group and the Albendazole® group (only one concentration was utilized, Table 1). The treatments encompassed AgNPs, AgNPs prepared with myrrh, myrrh (extract and essential oil), and Albendazole®. During the study, the treatment for the intestinal phase commenced on the third day post-infection and continued for three consecutive days. During this treatment phase, the objective is to impede the maturation of adult worms and prevent the subsequent migration of larvae, effectively hindering the progression of the life cycle (Ding et al., 2017). All treatments during this phase were administered orally. In contrast, the muscular phase was addressed through two administration routes. AgNPs and myrrh AgNPs were administered via intraperitoneal injections. Additionally, myrrh (extract and essential oil) and Albendazole® were administered orally. These treatment regimens were administered for seven consecutive days, commencing on the 31st day postinfection. Administering drugs on day 31 strategically aligns with the matured state of encapsulation, aiming to disrupt this crucial phase in the parasite's life cycle. Targeting this encapsulation phase intends to hinder the completion of the life cycle, minimizing the potential for sustained infection and transmission (Marian et al., 2020). Albendazole® (Alzental) was obtained from the Egyptian International Pharmaceutical Industries. The mice have received 50 mg/kg orally (Sawatdee et al., 2019).

2.3 | Preparation of methanolic myrrh extract and AgNPs using myrrh extracts

The myrrh exudate was sourced from the stems of *Commiphora myrrha* and was acquired from a reputable herbal market in Assiut, Egypt. To validate its authenticity, the exudate underwent a comprehensive analysis utilizing a combination of physical features and established chemical testing protocols (Evans, 2002). The powdered myrrh (125 g) was macerated in 1 L of methanol for 24 h. The material was sonicated for an hour the following day in a Crest CP2600HT Ultrasonic

TABLE 1 The therapeutic concentrations of all groups.

		Intestinal phase	Muscular phase
Groups	Subgroups	Concentration	
I. Infected Untreated group	I	(–)	(–)
II. Infected treated with AgNPs	II a	30 µg/mL	30 µg/mL
	II b	40 µg/mL	40 µg/mL
III. Infected treated with myrrh AgNPs	III a	30 µg/mL	30 µg/mL
	III b	40 µg/mL	40 µg/mL
IV. Infected treated with myrrh crude extract	IV a	500 mg/kg	800 mg/kg
	IV b	800 mg/kg	1000 mg/kg
V. Infected treated with myrrh essential oil	V a	500 mg/kg	800 mg/kg
	V b	800 mg/kg	1000 mg/kg
VI. Infected treated with Albendazole®	VI	50 mg/kg	50 mg/kg

Abbreviation: AgNPs, silver nanoparticles.

Cleaner to speed up the extraction process. This process was done five times, and the resultant filtrate was vacuum concentrated at 40°C using a rotary evaporator each time. The dried residues were mixed to obtain 28 g of crude extract.

Myrrh essential oil was obtained through hydrodistillation by placing 250 g of finely powdered myrrh in a 2 L glass flask with 1 L of distilled water and using a Clevenger apparatus to condense the water vapor containing the essential oil. The resultant extract was then used to create AgNPs through a green synthesis method (Abd-Elrahman et al., 2020).

The process of preparing AgNPs involved the utilization of a green biosynthetic technique (Salaheldin et al., 2019). In this method, a solution of $AgNO_3$ (0.95 mM) in distilled water (20 mL) was combined with a portion of myrrh extract and stirred vigorously for an hour using a magnetic stirrer. The formation of AgNPs was indicated by the change in color from faint yellow to dark yellow and eventually green (Abd-Elrahman et al., 2021).

AgNPs were synthesized by reducing $AgNO_3$ solution with sodium borohydride ($NaBH_4$) in an aqueous solution, both purchased from Sigma–Aldrich (MO, USA). A large excess of $NaBH_4$ was used to stabilize the formed AgNPs, and an ice bath was employed to slow down the reaction and control particle size/shape. Polyvinyl pyrrolidone (PVP, M.wt 25 K) from Fisher Scientific (NJ, USA) was also used as a stabilizer to prevent AgNPs aggregation. The reaction resulted in the formation of spherical AgNPs (Abd-Elrahman et al., 2021).

2.4 | Parasitological assessment of the treatment efficacy

Six days after infection, mice were sacrificed to assess the effects of various treatments on adult worms during the intestinal phase. The count of adult worms present in the gut was identified and tallied (Attia et al., 2015). Similarly, mice belonging to group II were sacrificed on the 49th day after infection to evaluate the effects of these agents on larvae (Yadav, 2012). The mice carcass was minced, digested with artificial digestive fluid, sieved, and washed by repeated sedimentation in phosphate-buffered saline (PBS). These larvae were then counted using a stereomicroscope at a magnification of 40. The larval quantity was determined by multiplying the number of larvae found in 50 μL by 8000, resulting in the total number of larvae found in the carcass (Gottstein et al., 2009). The formula for determining the number of larvae per carcass is given as larvae/carcass = mean larvae in 50 μL × 8000 (Shoheib et al., 2013).

The efficiency of treatment was calculated using the following equation:

The efficacy of treatment (%) = 100 × (mean number recovered in controls − mean number recovered in treated mice)/mean number recovered in controls.

2.5 | Histological examination

To evaluate the histopathological changes, skeletal muscle samples were obtained from the diaphragm of mice that were sacrificed on the 49th-day postinfection in both control and treated groups. The specific choice of day 49 ensures a thorough evaluation of the treatment's effectiveness and its consequences on the host's muscular tissues (Park et al., 2018). This time point allows for a comprehensive examination of the histopathological alterations in the muscles, providing insights into the persistence or resolution of the infection and the potential impact of the treatment on the later stages of the parasite's life cycle (Fahmy & Diab, 2021).

Following collection, the samples were subjected to fixation in 10% neutral buffered formalin and dehydration in ascending concentrations of ethyl alcohol series. The tissue specimens were then embedded in paraffin and cut into 5 μm sections using a rotatory microtome (Slaoui & Fiette, 2011). The resulting sections were placed on glass slides, which were subsequently stained with hematoxylin and eosin before being analyzed for morphological changes using a light microscope (Olympus CX31, Tokyo, Japan) and captured on a camera (Olympus, Camedia C-5060, Tokyo, Japan) in the Photomicrograph Lab of the Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Assiut University.

2.6 | Evaluation of nanoparticle combinations cytotoxicity

The toxicity of combinations of nanoparticles was previously evaluated (Lee et al., 2018; Said et al., 2012). To conduct the evaluation, the liver, brain, lungs, and intestine were extracted from mice that were sacrificed on the 6th day for the intestinal phase and the 49th day for the muscular phase. The organs were weighed and preserved in a 10% formalin solution with a few drops of PBS. Tissue samples weighing 0.3 g were digested using a microwave digestion system with 3 mL of concentrated nitric acid. The oven temperature was increased from 20 to 150°C within 5 min, maintained at 150°C for 5 min, increased from 150 to 200°C within 3 min, and then maintained at 200°C for an hour. The concentrations of Ag in the digested fluid were analyzed using an atomic absorption spectrophotometer through a flameless method. The concentration of silver in the tissue was expressed as nanograms per gram of wet weight.

2.7 | Statistical analysis

Data coding and analysis were performed using the Social Sciences Statistical Analysis Software Package (SPSS) version 20 from IBM Corp. in Armonk, NY, USA. Mean and standard deviation were used to express quantitative variables, while frequencies (number of cases) and relative frequencies (percentages) were used for categorical variables. To compare groups, ANOVA with post hoc tests were conducted for normally distributed quantitative variables, and the percentage of reduction between treated and control groups was calculated. The independent *t*-test was used to determine the significance of differences between groups, with statistical significance set at $p < .05$.

3 | RESULTS

3.1 | Parasitological assessment of different treatment agents

Regarding the effect of different treatments on the intestinal phase compared to the infected untreated group, the results showed that the crude extract of myrrh had the highest anthelmintic activity against *T. spiralis* adult worms, followed by AgNPs. Moreover, the AgNPs prepared with myrrh had a closely similar effect to that detected by crude extract of myrrh and AgNPs. However, albendazole exhibited a mortality rate of 80.31% at a concentration of 50 mg/kg. The lowest percentage of adult worms reduction was observed in the group treated with myrrh essential oil. All treated groups showed a significant reduction in the mean number of adult worms compared with the control group ($p < .001$), which had an average number of adult worm of 215 ± 12.2. Importantly, no significant differences were detected between different concentrations of AgNPs ($p = .102$), AgNPs prepared with myrrh ($p = .089$), and myrrh crude extract ($p = .091$). However, a significant difference was found between doses of 500 and 800 mg/kg of myrrh essential oil ($p < .001$; Table 2).

The outcome indicates that there is no notable contrast among the high concentrations of treatments, but a significant difference exists between 40 μ/mL AgNPs, myrrh crud extract of 800 mg/kg, and essential oil of myrrh at 800 mg/kg with ($p < .001$; Table 3).

Regarding their impact on the muscular phase, AgNPs, AgNPs combined with myrrh, myrrh, and albendazole revealed similar results, with the essential oil of myrrh following closely behind. Compared to the mean number of *Trichinella* sp. observed in the untreated group of infected individuals ($269.3 \pm 35.9 \times 10^3$), the highest percentage of adult worms reduction was observed in larval population with AgNPs combined with myrrh at concentrations of 30 and 40 μ/mL, which is almost identical to the reduction seen with AgNPs at concentrations of 30 and 40 μ/mL. Albendazole produced a reduction of 89.1% at a concentration of 50 mg/kg, followed by crude extract of myrrh at concentrations of 800 and 1000 mg/kg. The lowest reduction percentage was observed in the larval population treated with volatile oils of myrrh at concentrations of 800 and 1000 mg/kg. All treated groups showed a significant decrease in the mean number of larvae compared to the control group ($p < .001$). No significant difference was observed between varying concentrations of each treatment. Moreover, high concentrations of each treatment also did not exhibit any significant difference (Table 4).

3.2 | Histopathological assessment of therapeutic effect

In the examined muscles, Subgroup I (infected untreated mice; Figure 1a) showed a large number of encysted *T. spiralis* larvae diffused throughout the sarcoplasm, accompanied by a significant presence of chronic inflammatory cells such as lymphocytes, plasma cells, eosinophils, and histiocytes. These inflammatory cells infiltrated the muscle bundles and surrounded the encysted larvae, resulting in

TABLE 2 Percentages of adult worms' reduction in the intestinal phase of all infected treated groups, compared to the infected untreated group.

	II		III		IV		V		VI
Subgroup	**(a)**	**(b)**	**(a)**	**(b)**	**(a)**	**(b)**	**(a)**	**(b)**	
Treatment Concentration	30 μ/ml	40 μ/ml	30 μ/ml	40 μ/ml	500 mg/kg	800 mg/kg	500 mg/kg	800 mg/kg	50 mg/kg
No. of adult worm (Mean ± SEM)	36.30 ± 19.4*	32.33 ± 2.1*	42.33 ± 12.2*	37.60 ± 16.2*	33.00 ± 13.4*	28.67 ± 15.6*	71.60 ± 16.8*	47.00 ± 25.8*	42.33 ± 17.8*
Reduction (%)	83.12	84.96	80.31	82.51	84.65	86.66	66.69	78.14	80.31
p-value	IIa versus IIb = .102		IIIa versus IIIb = .089		IVa versus IVb = .091		Va versus Vb <.001		

*Statistically significant ($p < .001$) compared to the control group "Subgroup I".

TABLE 3 Comparison between high concentrations of each treatment in the intestinal phase.

Subgroup	IIb	IIIb	IVb	Vb	VI
Treatment Concentration	40 μ/mL	40 μ/mL	800 mg/kg	800 mg/kg	50 mg/kg
No. of adult worm (Mean ± SEM)	32.33 ± 2.1	37.60 ± 16.2	28.67 ± 15.6	47.00 ± 25.8	42.33 ± 17.8
Reduction (%)	84.96	82.51	86.66	78.14	80.31
p-value	IIb versus IIIb = .068	IIb versus IVb = .041	IIb versus Vb <.001	IVb versus Vb <.001	IIIb versus IVb = .044
	IIIb versus Vb = .001	IIIb versus VI = .003	IVb versus Vb <.001	IVb versus VI = .048	Vb versus VI = .231

TABLE 4 Percentages of reduction of larvae in the muscular phase of all infected treated groups, compared to the infected untreated group.

	II		III		IV		V		VI
Subgroup	**(a)**	**(b)**	**(a)**	**(b)**	**(a)**	**(b)**	**(a)**	**(b)**	
Treatment Concentration	30 μ/mL	40 μ/mL	30 μ/mL	40 μ/mL	800 mg/kg	1000 mg/kg	800 mg/kg	1000 mg/kg	50 mg/kg
No. of larva (Mean ± SEM) $\times 10^3$	21.3 ± 1.9*	20 ± 3.3*	20 ± 3.3*	17.3 ± 1.9*	61.3 ± 15.1*	38.7 ± 9.9*	65.3 ± 22.2*	48 ± 4.9*	29.3 ± 13.1*
Reduction (%)	92.07	92.57	92.57	93.5	77.22	85.64	75.74	82.18	89.1
p-value	IIa versus IIb = .687		IIIa versus IIIb = .234		IVa versus IVb = .003		Va versus Vb = .034		

*Statistically significant ($p < .001$) compared to the control group "Subgroup I".

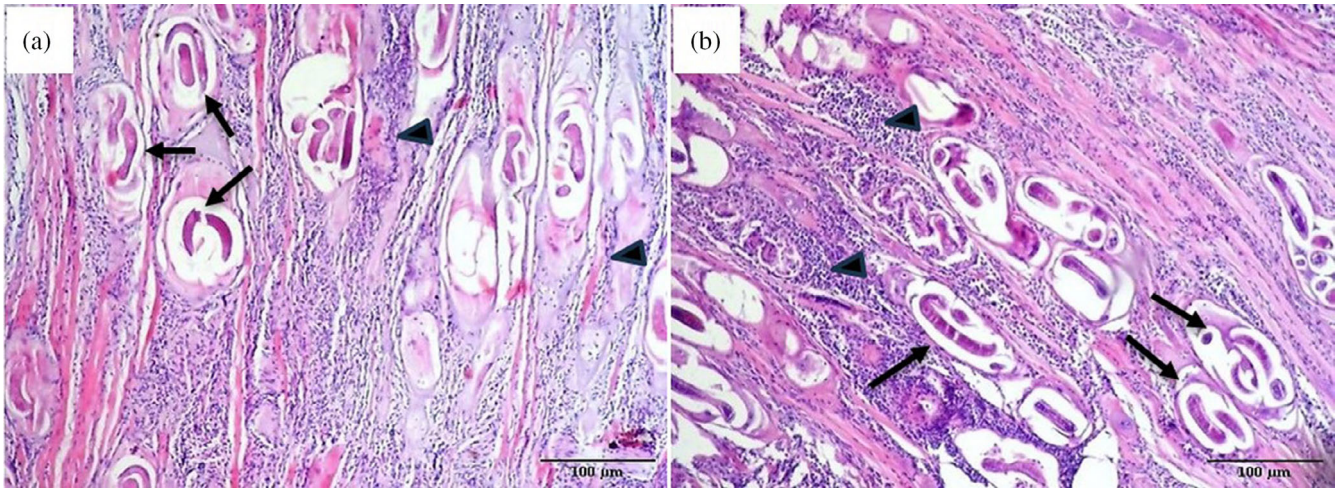

FIGURE 1 Histopathological assessment of *Trichinella spiralis* infection in skeletal muscle in response to volatile oil stained with H&E. (a) Skeletal muscle section from the diaphragm of infected, non-treated group showing a *T. spiralis* larva (arrows) associated with massive inflammatory cellular infiltration (arrow heads; bar = 100 μm). (b) Skeletal muscle section from the infected group treated with volatile oil showed the highest number of *T. spiralis* larva among all treated ones (arrows), with increased infiltration of inflammatory cells (arrow heads; bar = 100 μm).

muscle fiber necrosis. Subgroup V (Figure 1b), which received volatile oils of myrrh, was the least effective in reducing the larval burden. It exhibited multiple encysted larvae of *T. spiralis* located between the muscle fibers, along with degenerative changes and increased infiltration of inflammatory cells. The capsules of most larvae in this subgroup appeared thick and intact, with a smaller area of muscle fiber necrosis.

In contrast, the muscular sections from Subgroup II (Figure 2a), which received AgNPs, and Subgroup IV (Figure 2b), which received AgNPs prepared with myrrh, showed a significantly lower number of encysted larvae. These sections exhibited degenerative changes, thinning, and splitting of the capsules into thin layers, breakdown areas, vacuolization, and less lymphocytic infiltration around the larvae. There was also a smaller area of muscle fiber necrosis.

Muscle sections from Subgroup IV (Figure 3a) treated with myrrh, and Subgroup VI (Figure 3b) treated with albendazole, had similar results. However, albendazole appeared to be more effective in causing larval death, as evidenced by the precipitation of calcium bodies around the deceased larvae.

3.3 | Evaluation of nanoparticle-associated cytotoxicity

On the third day of treatment, organs were collected and analyzed for the presence of AgNPs. The liver, brain, lung, and intestine were found to have varying levels of nanoparticle precipitation. A significant difference was observed between myrrh-prepared AgNPs and regular AgNPs in the liver and brain ($p < .001$), but not in the lung and intestine during the intestinal phase of the study (Table 5).

During the muscular phase, organs were gathered on the 49th day, 10 days after the end of the treatment. The highest amount of AgNPs precipitation was detected in the liver, followed by the brain, and lung. A significant difference ($p < .001$) was observed between AgNPs prepared with myrrh and those without in the liver, brain, and lung (Table 6).

4 | DISCUSSION

This study revealed that both AgNPs and AgNPs prepared with myrrh were successful in treating both the intestinal and muscular stages of

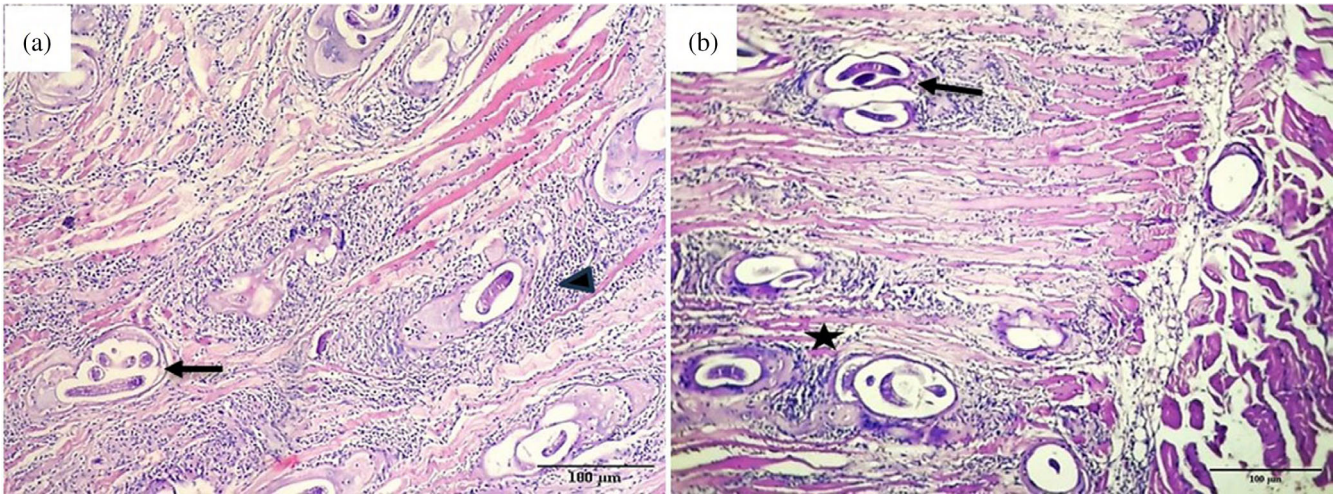

FIGURE 2 Histopathological assessment of *Trichinella spiralis* infection in skeletal muscle in response to silver nanoparticles (AgNPs) and AgNPs with myrrh stained with H&E. (a) Skeletal muscle section from the infected group, treated with AgNPs showed much fewer numbers of encysted larvae (arrow) and less lymphocytic infiltration (arrow head; bar = 100 µm). (b) Skeletal muscle section from the infected group treated with AgNPs prepared with myrrh, showed much fewer numbers of encysted larvae (arrow), and most of them showed degenerative changes (star; bar = 100 µm).

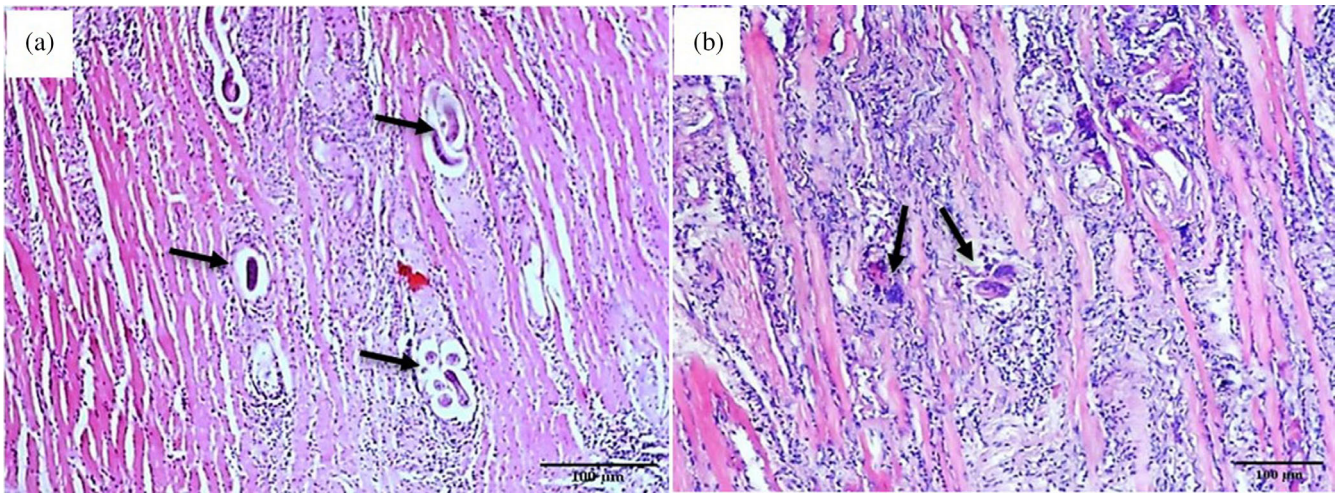

FIGURE 3 Histopathological assessment of *Trichinella spiralis* infection in skeletal muscle in response to myrrh and albendazole stained with H&E. (a) Skeletal muscle section obtained from the infected group treated with myrrh showed a lower number of *T. spiralis* larvae (arrows; bar = 100 µm). (b) Skeletal muscle section from the infected group treated with albendazole showed the same effect observed in the infected group treated with myrrh, with precipitation of calcium bodies around the deceased larvae (arrows; bar = 100 µm).

TABLE 5 Concentration of silver nanoparticles (AgNPs) in tissue during intestinal phase.

Organ	Conc. Of AgNPs (ppm)	Conc. of myrrh-AgNPs (ppm)	*p* value
Liver	0.075 ± 0.01	0.32 ± 0.01	< .001
Brain	0.02 ± 0.001	0.305 ± 0.08	< .001
Lung	0.025 ± 0.004	0.095 ± 0.01	= .003
Intestine	0.01 ± 0.002	0.055 ± 0.001	= .011

Note: *p*-value <.001, statistically significant compared to the corresponding value in the infected untreated group (Subgroup I).

T. spiralis. These results were similar to the effects of albendazole, while myrrh volatile oil showed less effectiveness against the parasite. All groups that received treatment experienced a significant decrease in the average number of adults and larvae compared with the control group ($p < .001$). The efficacy of AgNPs was observed at concentrations of 30 and 40 µg/mL, with no notable difference between them.

TABLE 6 Concentration of Silver nanoparticles (AgNPs) in tissue during muscular phase.

Organ	Conc. of AgNPs (ppm)	Conc. of myrrh-AgNPs (ppm)	*p* value
Liver	0.285 ± 0.02	1.225 ± 0.05	< .001
Brain	0.015 ± 0.001	0.885 ± 0.03	< .001
Lung	0.0	0.1 ± 0.002	< .001

Note: *p*-value <.001, statistically significant compared to the corresponding value in the infected untreated group (Subgroup I).

At a concentration of 40 μg/mL, there was an 84.96% reduction in adults and a 92.57% reduction in larvae. These findings contradict the results reported in a previous study (El-Melegy et al., 2019), which showed low efficacy (38.46%) against the muscular phase. Regarding AgNPs prepared with myrrh, this study verified that, at a concentration of 40 μg/mL yielded an 82.51% reduction in adult worm count and a 93.5% reduction in larvae count, another study proved complete healing of lesions in BALB mice infected with *Leishmania major* after applying AgNPs from myrrh for 21 days (Al Olayan et al., 2017). Additionally, El-Sherbiny et al. (2013) demonstrated high antibacterial activity of AgNPs prepared with myrrh against *Bacillus thuringiensis* and *Pseudomonas aeruginosa* bacteria compared with myrrh extract.

In this study, the effectiveness of myrrh extract against *T. spiralis* was demonstrated. At a concentration of 800 mg/kg, it resulted in an 86.66% reduction in the adult stage of the worms. Additionally, at a concentration of 1000 mg/kg, it showed 85.64% reduction in the larval stage of the worms. These results were consistent with findings from another study (Attia et al., 2015). However, results of the following research (Basyoni & El-Sabaa, 2013) recorded a lower reduction rate, which may be due to differences in dosages and schedules used in the study. Treating with myrrh volatile oil exhibited lower efficacy against *T. spiralis*, with a percent of adult reduction of 78.14% and a percent of larva reduction of 82.18%. There was a significant difference between the effectiveness of myrrh volatile oil at concentrations of 500 and 800 mg/kg ($p < .001$). Albendazole had good antiparasitic activity producing 80.31% and 89.1% reduction in the count of adult worms and larvae, respectively. The study found an increase in the percentage reduction of the muscular stage compared with the adult stage in all treated groups.

AgNPs prepared with myrrh were found to be effective against encysted larvae of *T. spiralis* in muscular sections, as indicated by a lower number of larvae. This finding is consistent with a previous study (El-Melegy et al., 2019), that reported a reduction in larvae count and thinning of larvae capsule, as well as increased necrosis of larvae internal structure. Similarly, myrrh and albendazole showed similar results, with albendazole also causing the precipitation of calcium bodies, which aligns with findings from another study (Attia et al., 2015) that reported degeneration and damage to the capsule of encysted larvae. Additionally, Basyoni & El-Sabaa (2013) reported similar results. The least effect was observed in the group that received myrrh volatile oil, where multiple encysted larvae were seen between muscle fibers with degenerative changes and heavier inflammatory cellular infiltration surrounding them. This finding contradicts the results presented in a previous study (Aboulaila et al., 2020), which demonstrated that myrrh oil effectively inhibits the growth of bovine and equine piroplasm.

A concentration of 40 μg/mL of AgNPs (1.6 mg/kg) was utilized for 3 days in the intestinal phase and 7 days in the muscular phase. This concentration is similar to that used by Said et al. (2012), who treated *Giardia lamblia* with 50 μg/mL per mouse. All silver values detected in different tissues were found to be within the safe range, which is consistent with the previous findings (Kim et al., 2010; Said et al., 2012), where no observable adverse effects were reported when using AgNPs at a dose of 30 mg/kg, which is several times higher than the dose used in this study. The concentration of AgNPs in the organs of the muscular group (sacrificed 10 days after treatment cessation) was slightly higher than that of the intestinal phase group (sacrificed 1 day after treatment cessation), indicating continuous accumulation of Ag even after exposure termination. This finding is consistent with observation of Lee et al. (2018) who reported that Ag concentration in the brain of the recovery group was slightly higher than that of the 4-week administration group. Our study revealed that nanoparticles produced through green methods are more toxic than those obtained through nongreen methods ($p < .001$). However, their toxicities were generally within safe limits, implying that toxicity increases with increasing nanoparticle concentration and prolonged exposure.

Cytotoxicity assessments of AgNPs precipitation were highest in the liver, followed by the brain, lungs, and intestine. This aligns with previous researches (Lee et al., 2018; Loeschner et al., 2011), which also found high levels of silver distribution in the liver and intestine. However, another study (Kim et al., 2010) identified higher concentrations of silver in the liver, lungs, and brain. These findings contradict those of Said et al. (2012), who observed that silver distribution was highest in the intestine, followed by the kidneys, liver, and lungs; no silver was detected in the brain.

5 | CONCLUSION

In conclusion, the present study's findings suggest that AgNPs, particularly those prepared with myrrh, have therapeutic potential in treating *T. spiralis* infection, both in the intestinal and muscular phases. The antiparasitic effect was evidenced by a significant reduction in the number of larvae and adults, as well as histopathological improvements, with no significant toxicity detected in the organs examined. Plant-based green synthesis of nanoparticles appears to be a promising approach for increasing efficacy and minimizing adverse effects in

the treatment of parasitic infections. Further research, particularly clinical trials, is required to confirm the safety and efficacy of these nanoparticles in the treatment of *T. spiralis* infection.

AUTHOR CONTRIBUTIONS

Nahed A. Elossily: Conceptualization; supervision; methodology; data curation; validation; formal analysis; investigation; writing – review and editing. **Salwa M. Abd-ELrahman:** Conceptualization; methodology; data curation; investigation; validation; formal analysis; supervision; project administration; writing – original draft. **Abeer A. Khedr:** Methodology; data curation; investigation; validation; formal analysis; writing – original draft. **Ahmed Kamal Dyab:** Conceptualization; supervision; project administration; writing – review and editing; methodology; data curation; formal analysis; validation; investigation. **Abeer E. Mahmoud:** Writing – original draft; conceptualization; supervision; methodology; data curation; formal analysis; validation; investigation. **Shaymaa M. Mohamed:** Methodology; data curation; formal analysis; validation; investigation. **Ahmed M. Abd Elrahman:** Methodology; data curation; formal analysis; validation; investigation. **Fahd M. Alsharif:** Methodology; data curation; formal analysis; validation; investigation. **Reem M. Alsaadawy:** Methodology; data curation; formal analysis; validation; investigation. **Ramy K. A. Sayed:** Methodology; data curation; formal analysis; validation; investigation; writing – review and editing. **Mervat M. Khalifa:** Methodology; data curation; formal analysis; validation; investigation.

ACKNOWLEDGMENTS

The authors are grateful to Prof. Dr. Sary Khaliel Naser, Professor and head of pathology Department, Faculty of Veterinary Medicine, Assiut University, for his help with histopathological examination.

FUNDING INFORMATION

This research had no external funding.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Ramy K. A. Sayed https://orcid.org/0000-0003-4467-6742

REFERENCES

Abd-Elrahman, S. M., Dyab, A. K., Mahmoud, A. E., Alsharif, F. M., Mohamed, S. M., Abomughaid, M. M., & Elossily, N. A. (2021). Influence of chemically and biosynthesized silver nanoparticles on in vitro viability and infectivity of Trichinella spiralis muscle larvae. *Annals of Parasitology*, *67*, 591–602.

Abd-Elrahman, S. M., Dyab, A. K., Mahmoud, A. E., Mostafa, S. M., & Elossily, N. A. (2020). Antiparasitic activity of myrrh crude extract and myrrh volatile oil compared to albendazole against Trichinella spiralis muscular larvae in vitro. *Journal of the Egyptian Society of Parasitology*, *50*, 307–314.

Abou Rayia, D., Othman, A., Harras, S., Helal, D., Dawood, L., & Soliman, S. (2022). Bevacizumab: A new take on therapy of muscle phase of Trichinella spiralis infection. *Acta Tropica*, *230*, 106409.

Aboulaila, M., El-Sayed, S. A. E., Omar, M. A., Al-Aboody, M. S., Aziz, A. R. A., Abdel-Daim, M. M., Rizk, M. A., & Igarashi, I. (2020). Myrrh oil in vitro inhibitory growth on bovine and equine piroplasm parasites and *Babesia microti* of mice. *Pathogens*, *9*, 173.

Ahmad, A. A., Maurice, M. N., Monib, M. E.-S. M., Soliman, M., Al-Thagfan, S. S., & Huseein, E. A. M. (2023). Eugenol essential oil and nanoemulsion as antihydatic agents with antifibrotic and immunomodulatory effects in cystic echinococcosis. *Tropical Medicine and Infectious Disease*, *8*, 253.

Al Olayan, E. M., Awad, M. A. G., Siddiqui, M. I., & Merghani, N. M. (2017). *Method for preparing noble metal nanoparticles from myrrh*. Google Patents.

Allam, A. F., Mostafa, R. A., Lotfy, W., Farag, H. F., Fathi, N., Moneer, E. A., & Shehab, A. Y. (2021). Therapeutic efficacy of mebendazole and artemisinin in different phases of trichinellosis: A comparative experimental study. *Parasitology*, *148*, 630–635.

Attia, R. A., Mahmoud, A. E., Farrag, H. M. M., Makboul, R., Mohamed, M. E., & Ibraheim, Z. (2015). Effect of myrrh and thyme on Trichinella spiralis enteral and parenteral phases with inducible nitric oxide expression in mice. *Memórias Do Instituto Oswaldo Cruz*, *110*, 1035–1041.

Ayipo, Y. O., Bakare, A. A., Badeggi, U. M., Jimoh, A. A., Lawal, A., & Mordi, M. N. (2022). Recent advances on therapeutic potentials of gold and silver nanobiomaterials for human viral diseases. *Current Research in Chemical Biology*, *2*, 100021.

Basyoni, M. M., & El-Sabaa, A. A. (2013). Therapeutic potential of myrrh and ivermectin against experimental Trichinella spiralis infection in mice. *The Korean Journal of Parasitology*, *51*, 297–304.

Bergal, A., Matar, G. H., & Andaç, M. (2022). Olive and green tea leaf extracts mediated green synthesis of silver nanoparticles (AgNPs): Comparison investigation on characterizations and antibacterial activity. *BioNanoScience*, *12*, 307–321.

Bruna, T., Maldonado-Bravo, F., Jara, P., & Caro, N. (2021). Silver nanoparticles and their antibacterial applications. *International Journal of Molecular Sciences*, *22*, 7202.

Chouhan, S., & Guleria, S. (2020). Green synthesis of AgNPs using *Cannabis sativa* leaf extract: Characterization, antibacterial, anti-yeast and α-amylase inhibitory activity. *Materials Science for Energy Technologies*, *3*, 536–544.

Dara, P. K., Mahadevan, R., Digita, P., Visnuvinayagam, S., Kumar, L. R., Mathew, S., Ravishankar, C., & Anandan, R. (2020). Synthesis and biochemical characterization of silver nanoparticles grafted chitosan (chi-Ag-NPs): In vitro studies on antioxidant and antibacterial applications. *SN Applied Sciences*, *2*, 1–12.

Ding, J., Bai, X., Wang, X., Shi, H., Cai, X., Luo, X., Liu, M., & Liu, X. (2017). Immune cell responses and cytokine profile in intestines of mice infected with *Trichinella spiralis*. *Frontiers in Microbiology*, *8*, 2069.

Duignan, P. (2023). Aquatic Mammals. In *Pathology and Epidemiology of Aquatic Animal Diseases for Practitioners* (pp. 214–352). Wiley.

El-Melegy, M. A., Ghoneim, N. S., EL-Dien, N. M. N., & Rizk, M. S. (2019). Silver nano particles improve the therapeutic effect of mebendazole treatment during the muscular phase of experimental trichinellosis. *Journal of American Science*, *15*, 34–46.

El-Sherbiny, I. M., Salih, E., & Reicha, F. M. (2013). Green synthesis of densely dispersed and stable silver nanoparticles using myrrh extract and evaluation of their antibacterial activity. *Journal of Nanostructure in Chemistry*, *3*, 1–7.

Evans, W. C. (2002). *Trease and Evans pharmacognosy* (p. 21). Elsevier Health Sciences.

Fahmy, A., & Diab, T. (2021). Therapeutic efficacy of Albendazole and Mefloquine alone or in combination against early and late stages of infection in mice. *Helminthologia*, *58*, 179–187.

Gabriël, S., Dorny, P., Saelens, G., & Dermauw, V. (2023). Foodborne parasites and their complex life cycles challenging food safety in different food chains. *Food*, *12*, 142.

Gomez-Morales, M. A., Cherchi, S., & Ludovisi, A. (2022). Serological testing for trichinella infection in animals and man: Current status and opportunities for advancements. *Food and Waterborne Parasitology*, *27*, e00165.

Gottstein, B., Pozio, E., & Nockler, K. (2009). Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clinical Microbiology Reviews*, *22*, 127–145.

Guo, K. X., Bai, Y., Ren, H. N., Sun, X. Y., Song, Y. Y., Liu, R. D., Long, S. R., Zhang, X., Jiang, P., & Wang, Z. Q. (2020). Characterization of a *Trichinella spiralis* aminopeptidase and its participation in invasion, development and fecundity. *Veterinary Research*, *51*, 1–17.

Khalifa, M. M., Ramadan, R. M., Youssef, F. S., Auda, H. M., El-Bahy, M. M., & Taha, N. M. (2023). Trichinocidal activity of a novel formulation of curcumin-olive oil nanocomposite in vitro. *Veterinary Parasitology, Regional Studies and Reports*, *41*, 100880.

Kim, Y. S., Song, M. Y., Park, J. D., Song, K. S., Ryu, H. R., Chung, Y. H., Chang, H. K., Lee, J. H., Oh, K. H., & Kelman, B. J. (2010). Subchronic oral toxicity of silver nanoparticles. *Particle and Fibre Toxicology*, *7*, 1–11.

Lee, J. H., Sung, J. H., Ryu, H. R., Song, K. S., Song, N. W., Park, H. M., Shin, B. S., Ahn, K., Gulumian, M., Faustman, E. M., & Yu, I. J. (2018). Tissue distribution of gold and silver after subacute intravenous injection of co-administered gold and silver nanoparticles of similar sizes. *Archives of Toxicology*, *92*, 1393–1405.

Loeschner, K., Hadrup, N., Qvortrup, K., Larsen, A., Gao, X., Vogel, U., Mortensen, A., Lam, H. R., & Larsen, E. H. (2011). Distribution of silver in rats following 28 days of repeated oral exposure to silver nanoparticles or silver acetate. *Particle and Fibre Toxicology*, *8*, 18.

Longo, L. D., Gheorghe, C. P., & Goyal, R. (2014). Dietary and hypoxic protocols that alter placental gene expression in response to maternal stress. In *The guide to investigation of mouse pregnancy* (pp. 761–762). Elsevier.

Lopez-Cauce, B., Urquia, A., Menchen, L., Homma, K., Bolas-Fernandez, F., Garcia-Rodriguez, J. J., & Puerto, M. (2022). Lentinula edodes extract increases goblet cell number and Muc2 expression in an intestinal inflammatory model of *Trichinella spiralis* infection. *Biomedicine & Pharmacotherapy*, *150*, 112937.

Lupşe, M., Ionică, A. M., Flonta, M., Rus, M. A., & Briciu, V. (2023). Retrospective survey of human Trichinellosis in a Romanian infectious diseases hospital over a thirty-year interval—The never-ending story. *Pathogens*, *12*, 369.

Marian, I., Ionică, A. M., Deak, G., Ursache, T., Lefkaditis, M., Gherman, C. M., & Mihalca, A. D. (2020). The effect of *Trichinella spiralis* on muscular activity of experimentally infected mice. *Parasitology International*, *76*, 102032.

Mehwish, H. M., Liu, G., Rajoka, M. S. R., Cai, H., Zhong, J., Song, X., Xia, L., Wang, M., Aadil, R. M., Inam-UR-Raheem, M., Xiong, Y., Wu, H., Amirzada, M. I., Zhu, Q., & He, Z. (2021). Therapeutic potential of *Moringa oleifera* seed polysaccharide embedded silver nanoparticles in wound healing. *International Journal of Biological Macromolecules*, *184*, 144–158.

Park, M.-K., Kang, Y.-J., Jo, J.-O., Baek, K.-W., Yu, H.-S., Choi, Y. H., Cha, H.-J., & Ock, M. S. (2018). Effect of muscle strength by *Trichinella spiralis* infection during chronic phase. *International Journal of Medical Sciences*, *15*, 802–807.

Said, D., Elsamad, L., & Gohar, Y. (2012). Validity of silver, chitosan, and curcumin nanoparticles as anti-Giardia agents. *Parasitology Research*, *111*, 545–554.

Salaheldin, A., El-Chaghaby, G., & el-sherbiny, M. (2019). Green synthesis of silver nanoparticles using Portulacaria afra plant extract: Characterization and evaluation of its antibacterial, anticancer activities. *Novel Research in Microbiology Journal*, *3*, 215–222.

Salleh, A., Naomi, R., Utami, N. D., Mohammad, A. W., Mahmoudi, E., Mustafa, N., & Fauzi, M. B. (2020). The potential of silver nanoparticles for antiviral and antibacterial applications: A mechanism of action. *Nanomaterials*, *10*, 1566.

Sawatdee, S., Atipairin, A., Sae Yoon, A., Srichana, T., Changsan, N., & Suwandecha, T. (2019). Formulation development of albendazole-loaded self-microemulsifying chewable tablets to enhance dissolution and bioavailability. *Pharmaceutics*, *11*, 134.

Shoheib, Z. S., Ashour, D. S., Soliman, N. A., & Shafik, N. M. (2013). Infectivity of ivermectin-treated *Trichinella spiralis* larvae: A new challenge for control. *Parasitologists United Journal*, *6*, 193–204.

Slaoui, M., & Fiette, L. (2011). Histopathology procedures: From tissue sampling to histopathological evaluation. *Methods in Molecular Biology*, *691*, 69–82.

Tortella, G., Rubilar, O., Durán, N., Diez, M., Martínez, M., Parada, J., & Seabra, A. (2020). Silver nanoparticles: Toxicity in model organisms as an overview of its hazard for human health and the environment. *Journal of Hazardous Materials*, *390*, 121974.

Yadav, A. K., & Temjenmongla. (2012). Efficacy of Lasia spinosa leaf extract in treating mice infected with *Trichinella spiralis*. *Parasitology Research*, *110*, 493–498.

How to cite this article: Elossily, N. A., Abd-ELrahman, S. M., Khedr, A. A., Dyab, A. K., Mahmoud, A. E., Mohamed, S. M., Abd Elrahman, A. M., Alsharif, F. M., Alsaadawy, R. M., Sayed, R. K. A., & Khalifa, M. M. (2024). Light microscopical and parasitological analyses revealed the beneficial effects of silver nanoparticles and various myrrh extracts against *Trichinella spiralis* infection in mice. *Microscopy Research and Technique*, 1–10. https://doi.org/10.1002/jemt.24542