Benha Veterinary Medical Journal 41 (2021) 144-147

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

In vitro anthelmintic effects of iron oxide and zinc oxide nanoparticles against *Fasciola Spp.* in Dakhla Oasis, Egypt.

Ahmed, I Hassan[1], Mohamed, Y Ramdan[2], Omar, S.F. A[1] and Lubna, M Elkabawy[2]

[1] *Agriculture Research Center, Animal Health Research Institute, Egypt.*

[2] *Parasitology Department, Fac. Vet. Med. Benha University, Egypt.*

ARTICLE INFO

Keywords

Fasciola spp

Infection

FeO

ZnO

Nanoparticle

SEM

Dakhla Oasis

Received 13/07/2021

Accepted 27/07/2021

Available On-Line 01/10/2021

ABSTRACT

In the present study, iron oxide (FeO) and zinc oxide (ZnO) nanoparticles were studied for their potential effect against *Fasciola spp.* in vitro. *Fasciola* adult worms were incubated for 24 h with three concentrations (0.004, 0.008 and 0.012% w/v) for both FeO and ZnO. The parasites motility and mortality were recorded at different times. The result showed that both nanoparticles could significantly reduce the worms' motility and increased their mortality compared to the control group. Moreover, the parasites were examined by high-resolution SEM which showed an upper body dislocation with swelling of the worm's body, roughness, hemorrhage, desquamation, and complete loss of the spine induced by 0.012% conc. of both FeO and ZnO nanoparticles was more than other concentrations. It can be concluded that FeO and ZnO nanoparticles had potent fasciocidal activity especially at 0.012% Conc of both FeO and ZnO nanoparticles.

1. INTRODUCTION

Fascioliasis is one of most important liver affections of herbivorous animals caused by *Fasciola* species. It found in large groups of animals depend on grazing

It is of a global distribution in a large group of grass-grazing animals (Soulsby, 1982). In Egypt, estimated losses due to animal fascioliasis of 190 million Egyptian pounds. Opio et al., (2021) in Uganda found that the slaughterhouse losses were estimated at 38 million due to elimination of the liver infected with *Fasciola*. Therefore, it is important to take the necessary preventive measures to control disease and elevate awareness among farmers and medical personnel due to the zoonotic aspect of fascioliasis (Abdelazeem et al., 2020).

In the absence of an effective vaccine, the only good option is chemotherapy to treat and combat parasitic diseases, but there are several studies of the emergence of anthelmintic resistance in parasites (Khan et al., 2015). Moreover, the residues of these agents in domestic animal products (milk and meat) can cause serious problems for humans. A survey in South Africa revealed that helminth resistance is in a very serious situation and some worms may not soon be controlled by current helminth treatment (Van Wky etal., 1999). Therefore, searching for modern agents for the treatment of *Fasciola* infestations with high parasite toxicity, lower resistance and residues is a matter of great interest to pharmacists and veterinarians.

Recently, metal oxide nanoparticles were used as antibacterial (Wang et al., 2012), antiviral (You et al., 2011), antifungal (Kim et al., 2012), antiprotozoal (Baiocco et al., 2010, Delavari, 2014) and anthelmintic (Khan etal., 2015). Since, zinc oxide was recognized for its safety from the five zinc compounds that are decided by the United State Food and Drug Administration (Premanathan et al., 2011) and also, iron oxide can be phagocyted by the spleen and this served as important agent to drug delivery. So, the current work aimed to study in vitro anthelmintic role of zinc oxide and iron oxide nanoparticles against *Fasciola spp.*infection.

2. MATERIAL AND METHODS

2.1. Chemicals and reagents

In (Ebdah center – Giza - Egypt), iron oxide (20–40 nm) and zinc oxide (20–30 nm) nanoparticles were prepared by chemical method (Laurent et al., 2008) and then used for preparation of three concentrations (0.004, 0.008 and 0.012% w/v)

2.2. Collection of flukes from infected liver

Out of 100 adult flukes were collected from infected liver of cattles at Mout main slaughterhouse, El- Dakhla province, El-WadiElgadid Governorate, Egypt. The adult flukes were transported to AHRI Dakhla laboratory for the study.

* Corresponding author: drahmed2034@yahoo.com

2.3. Preparation of different concentrations of ZnO and FeO nanoparticle

Concentration of 10 mg of both zinc oxide and iron oxide nano particles was used for preparation of stock solution using sonicator probe (Branson Sonifier, USA) at 30 W for 10 min. Then, three concentrations of each of the used nano particles (0.004, 0.008 and 0.012% w/v) were prepared using Ringer solution. Control group (Fasciola adult worms) treated with Ringer solution only without any concentration of ZnO and FeO nanoparticle.

2.4. Incubation of parasites with ZnO and FeO nanoparticles suspensions

Seven flasks of 100 ml labeled with different concentrations of the nano particles and control (six conc of nanoparticles + one control). Ten worms were transferred to each flask containing 70ml of each conc. and incubated for 24 h at 37c in 5% CO2 incubator. The worms were examined by light stereomicroscope at different time (3,6,12 and 24h) and the number of dead flukes was recorded according to (Dorostkar et al., 2017).

2.5. Scanning electron microscopy (SEM)

After 24 h of incubation, worms were preserved with 70% (Ethyl alcohol) and were transported to (Electron microscope unit) in Faculty of Medicine, Assiut University Worms were fixed with 4% (v/v) glutaraldehyde in PBS buffer (pH 7.4) for 24h at room temperature. Washing, dehydration and fixation of slides were carried out according **(**Keiser et al., 2006). Slides were examined by high resolution SEM.

3. RESULTS

3.1. Parasite mobility

The movement of worms are checked using a light stereomicroscope which represented in (Table 1). Where, 0.004% ZnO showed moderate mobile of *Fasciola spp.* after 24h of exposure. The same concentration of FeO showed moderate motility after 6h and a lower motility was reported after 12h of exposure. Using of 0.008% ZnO and FeO demonstrated low motility after 24h and 6h, respectively. Immobile *Fasciola spp*. (dead) were observed after exposure of these worms to 0.012% ZnO for 24h and 0.012% FeO for 12 h.

Table 1: - The invitro effect of FeO and ZnO nanoparticles on the mobility of *Fasciola spp.*

Parameter	Conc. %	Total worm used	Mortality rate, time (h)			
			3	6	12	24
FeO	0.004 %	10	$+++^{a}$	$++++^{a}$	$+++^{b}$	$+++^{b}$
	0.008 %	10	$++++^{a}$	$++++^{b}$	$++++^{b}$	$+++^{c}$
	0.012%	10	$+++^{b}$	$++^{c}$	$+^{d}$	….^{e}
Control	-	10	$++++^{a}$	$++++^{a}$	$++++^{a}$	$+++^{b}$
ZnO	0.004 %	10	$++++^{a}$	$++^{b}$	$++^{c}$	$++^{c}$
	0.008 %	10	$+++^{b}$	$++^{c}$	$++^{c}$	$+^{d}$
	0.012%	10	$++^{c}$	$+^{d}$	….^{e}	….^{e}

Values within a row and column carrying different superscript letter (a–e) denote significant differences (P 0.05). FeO = (Iron Oxide), ZnO = (Zinc Oxide).

3.2. The results of Scanning Electron Microscope

3.2.1. Control flukes

All Fasciola spp showed no damage of ventral or oral suckers (Fig 1A). The normal appearance of the tegmental architecture is shown at Figure 1B.

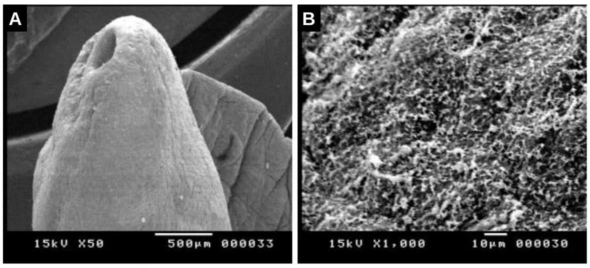


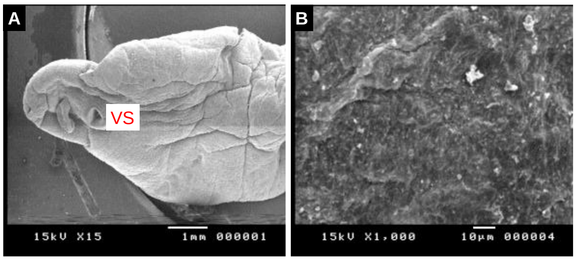
Fig (1): SEM of *Fasciola* control worm.

3.2.2. FeO treatment

3.2.2.1 FeO (0.004%)

SEM of *Fasciola* worms exposed to FeO (0.004%) (A) small widening of the ventral sucker (VS); (B) tegmental bulging and desquamated with loss of spines. (Fig2).

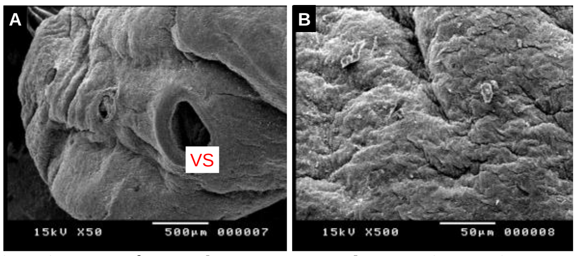
Fig. 2A. SEM of *Fasciola* worms exposed to FeO (0.004%). (A) Small widening of the ventral sucker (VS)



(Fig 2): SEM of *Fasciola* worms exposed to FeO (0.004%).

3.2.2.2. FeO (0.008%)

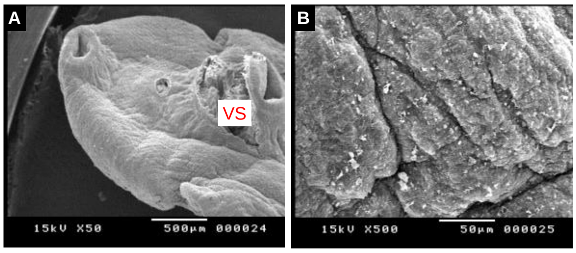
The result of FeO (.008%) Conc on worms (A) increase in widening of the ventral sucker (VS); (B)more tegmental bulging and desquamated with loss of spines. (Fig3).



(Fig 3): SEM of *Fasciola* worms exposed to FeO (0.008%).

3.2.2.3. FeO (0.0012)

Exposure of worms to FeO (0.012%) Conc. resulted in (A) deformed, swollen and damaged of ventral suckers (VS). (B) disruption, fissures on the tegument of flukes with complete loss of spines (Fig4).



(Fig4): SEM of *Fasciola* worms exposed to FeO (0.012%).

3.2.3. ZnO treatment

3.2.3.1 ZnO (0.004%)

SEM of *Fasciola* worms exposed to ZnO (0.004%) (A) small widening of the ventral sucker (VS). (B) tegmental bulging and desquamated with partial loss of spines. (Fig5).

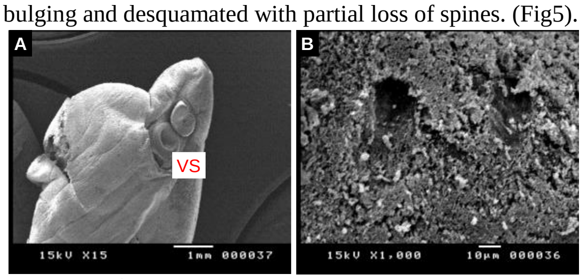


Fig (5): SEM of *Fasciola* worms exposed to ZnO (0.004%)

SEM of *Fasciola* worms exposed to ZnO (0.004%) (A) small widening of the ventral sucker (VS). (B) tegmental bulging and desquamated with partial loss of spines. (Fig5).

3.2.3.2. ZnO (0.008%)

SEM of *Fasciola* worms exposed to ZnO (0.008%) (A) increase in width of the ventral sucker (VS). (B) increased tegmental bulging, desquamation and fall of spines. (Fig 6)

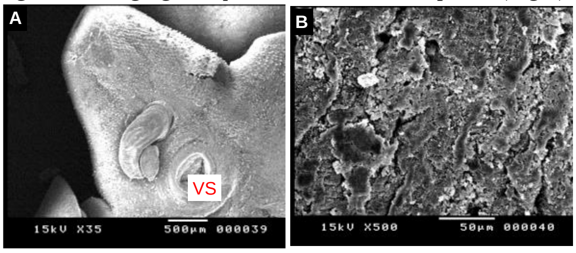


Fig (6): SEM of *Fasciola* worms exposed to ZnO (0.008%)

3.2.3.3. ZnO (0.012%)

SEM of *Fasciola* worms exposed to ZnO (0.0012%) **(**A) increase in widening and swelling of the ventral sucker (VS). (B) roughness, bleeding, and fissures on the tegmental surface with complete loss of spines (Fig7).

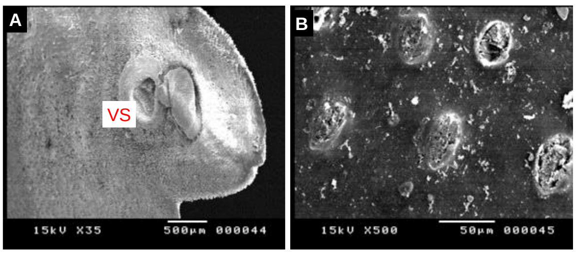


Fig (7): SEM of *Fasciola* worms exposed to ZnO (0.012%).

4. DISCUSSION

Regarding the increase of Fasciola resistance to chemical drugs, a new parasitic control strategy is needed to avoid the existing problems as many parasitic worms of veterinary importance are able to gradually develop anthelminthic resistance, because of their unique genetic traits. This has become a major global limitation in livestock production that can be seen as a serious risk to future output of grazing the animals. (Shalaby et al., 2013).

In the present work, the effects of zinc oxide and iron oxide nanoparticles on fascioliasis were evaluated. In previous years, several types of nanoparticles have been proposed as an antiparasitic agent (Delavari et al., 2014, Khan et al., 2015)

This study approved that both of the nanoparticles are effective on the worm, by increased mortality rate and decreased mobility of the parasite depending on time and concentration of nanoparticles. Although, it was reported that FeO nanoparticles are more potent than ZnO. This could be related to the type of the nanoparticles. Each nanoparticle has its own properties that can affect the nanotoxicity behaviors of nanoparticles (Chang et al., 2012). This result was in parallel with previous results of Dorostkar et al. (2017) who found that both of (ZnO)and (FeO) nanoparticles could significantly decrease *Toxocara vitulorum* mobility in vitro and (Baghbani et al., 2020) who studied in vitro effect zinc oxide nanoparticles (ZnO-NPs) on Teladorsagia circumcincta parasite and recorded that (ZnO-NPs) could extremely increase mortality rate of parasite.

SEM results showed the using of both ZnO and FeO nano particles caused widening in ventral sucker and tegmental disruption with roughness, bledding, sloughing and loss of spines which induced by 0.004% and 0.012% conc. of both FeO and ZnO nanoparticles. 0.012% conc was more effective than other concentrations. This result coincided with that of Gherbawy et al., (2013) who reported that silver nanoparticles (AgNBs) in combination with triclabendazole have potent effect on *Fasciola spp*. by decreasing egg hatchability. SEM showed perforation in egg due to effect of drug with (AgNBs) and (Morsy et al., 2019) determined the effect of ZnO nanoparticles (ZnO NPs) on nematode *P. equorum*. SEM showed that lips were wrinkled with irregularly arranged denticles, weathering of cuticle, bursts of cuticle layers, disruption of surface annulations and erosion of surface papillae of male around the cloacal opening.

In conclusions, ZnO and FeO nanoparticle will be used in treatment of fascioliasis in vivo because their potent fasciocidal activity and this represent useful tool in control of disease, especially in presence of drug resistance of parasite.

6. REFERENCES

1. Abdelazeem, A.G., Abdelaziz, A.R., Khalafalla, R.E., Mostafa, F.N. and Abushahba (2020): Prevalence and Phylogenetic analysis of *Fasciola* species in Upper Egypt Based on Ribosomal ITS-2 gene Sequencing. *EVMSPJ*, 16:142-158.
2. Baghbani , Z.; Esmaeilnejad, B. and Asri-Rezaei, S. (2020): Assessment of oxidative/nitrosative stress biomarkers and DNA damage in Teladorsagia circumcincta following exposure to zinc oxide nanoparticles. *Journal of Helminthology* , 94 , e115 doi.org/10.1017/S0022149X19001068.
3. Baiocco, P., Ilari, A., Ceci, P., Orsini, S., Gramiccia, M., Di Muccio, T. and Colotti, G (2010): Inhibitory effect of silver

nanoparticles on Trypanothione reductase activity and Leishmania infantum proliferation. *ACS Med. Chem. Lett,* 2(3): 230–233.

4. Chang, Y.-N., Zhang, M., Xia, L., Zhang, J., Xing, G. (2012): The toxic effects and mechanisms of CuO and ZnO nanoparticles. *Materials*, 5(12): 2850 – 2871.
5. Delavari, M., Dalimi, A., Ghaffarifar, F., Sadraei, J. (2014): In vitro study on cytotoxic effects of ZnO nanoparticles on promastigote and amastigote forms of Leishmania major. *Iran. J. Parasitol,* 9(1): 6–13.
6. Dorostkar, R.; Ghalavand, M.; Nazarizadeh, A.; Tat, M. and Hashemzadeh, M. S. (2017): Anthelmintic effects of zinc oxide and iron oxide nanoparticles against Toxocara vitulorum. *Int Nano Lett*, 7:157–164.
7. Gherbawy, Y. A.; Shalaby, I.M.; Abd El-sadek, M.S.; Elhariry, H.M. and Abdelilah, B.A. (2013): The Anti-Fasciolasis Properties of Silver Nanoparticles Produced by *Trichoderma harzianum* and Their Improvement of the Anti-Fasciolasis Drug Triclabendazole. *Int. J. Mol. Sci,* 14: 21887-21898.
8. Keiser, J., Xiao, S.H., Xue, J., Chang, Z.S., Odermatt, P., Tesana, S., Tanner, M., Utzinger, J., (2006): Effect of artesunate and artemether against Clonorchis sinensis and Opisthorchis viverrini in rodent models. *International Journal Antimicrobial Agents*, 28, 370–373.
9. Khan, Y.A., Singh, B.R., Ullah, R., Shoeb, M., Naqvi, A.H. and Abidi, S.M.A. (2015): Anthelmintic effect of biocompatible zinc oxidenanoparticles (ZnO NPs) on Gigantocotyle explanatum, aneglected parasite of Indian Water Buffalo. *PLoS ONE*, 10(7), e0133086.
10. Kim, S.W., Jung, J.H., Lamsal, K., Kim, Y.S., Min, J.S. and Lee, Y.S (2012): Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi. *Mycobiology*, 40(1): 53–58.
11. Laurent, S.; Forge, D. and Port, M. (2008): Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations and biological applications. Chem Rev, 108(6): 2064–2110.
12. Morsy, K.; Fahmy, S.; Mohamed, A.; Ali, S.; El–Garhy, M and Shazly, M. (2019): Optimizing and Evaluating the Antihelminthic Activity of the Biocompatible Zinc Oxide Nanoparticles Against the Ascaridid nNematode, Parascaris equorum In Vitro. *Acta Parasitologica*, 64(4): 873–886.
13. Opio, L.G., Abdelfattah, E.M., Terry, J., Odongo, S. and Okello, E. (2021): Prevalence of Fascioliasis and Associated Economic Losses in Cattle Slaughtered at Lira Municipality Abattoir in Northern Uganda. *Animals*, 11 (681):1-10.
14. Premanathan, M., Karthikeyan, K., Jeyasubramanian, K. and Manivannan, G. (2011): Selective toxicity of ZnO nanoparticles toward Gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation. Biol. Med, 7(2): 184–92.
15. Shalaby, H.A.: Anthelmintics resistance; how to overcome it? Iran. J. Parasitol. 8(1): 18–32.
16. Soulsby, E.J. (1982): Helminths, arthropods and domesticated animals. 7th Ed., Bailliere, Tindall and Cassell, London. pp. 40-52.
17. Van Wky, J., Stenson, M., Van der Merwe, J., Vorster, R. and Viljoen,P. (1999): Anthelmintic resistance in South Africa: surveys indicate an extremely serious situation in sheep and goat farming. Onderstepoort J. Vet. Res, 66(4): 273- 84.
18. Wang, C., Liu, L.-L., Zhang, A.-T., Xie, P., Lu, J. and Zou, X. T. (2012): Antibacterial effects of zinc oxide nanoparticles on Escherichia coli K 88. Afr. J. Biotechnol, 11(44):10248–10254.
19. You, J., Zhang, Y. and Hu, Z. (2011): Bacteria and bacteriophage inactivation by silver and zinc oxide nanoparticles. Colloids Surf, B 85(2): 161–167.