**Original Paper**

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

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**ARTICLE INFO**

**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 fasciosialsis 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 et al., 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 et al., 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 cattle at Mout main slaughterhouse, El- Dakhla province, El-WadilElgadid Governorate, Egypt. The adult flukes were transported to AHRI Dakhla laboratory for the study.

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2.3. Preparation of different concentrations of ZnO and FeO nanoparticles

Concentration of 10 mg of both zinc oxide and iron oxide nanoparticles 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 nanoparticles (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 nanoparticle suspensions and control (six conc of nanoparticles + one control). Ten worms were transferred to each flask containing 70 ml of each conc. and incubated for 24 h at 37°C in 5% CO2 incubator. The worms were examined by light stereomicroscope at different time (3, 6, 12 and 24 h) 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 24 h 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 24 h of exposure. The same concentration of FeO showed moderate motility after 6 h and a lower motility was reported after 12 h of exposure. Using of 0.008% ZnO and FeO demonstrated low motility after 24 h and 6 h, respectively. Immobile Fasciola spp. (dead) were observed after exposure of these worms to 0.012% ZnO for 24 h and 0.012% FeO for 12 h.

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Conc. %</th>
<th>Total worm used</th>
<th>Mortality rate, time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeO</td>
<td>0.004%</td>
<td>10</td>
<td>+++*</td>
</tr>
<tr>
<td></td>
<td>0.008%</td>
<td>10</td>
<td>+++*</td>
</tr>
<tr>
<td></td>
<td>0.012%</td>
<td>10</td>
<td>+++*</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>10</td>
<td>+++*</td>
</tr>
<tr>
<td>ZnO</td>
<td>0.004%</td>
<td>10</td>
<td>+++*</td>
</tr>
<tr>
<td></td>
<td>0.008%</td>
<td>10</td>
<td>+++*</td>
</tr>
<tr>
<td></td>
<td>0.012%</td>
<td>10</td>
<td>+++*</td>
</tr>
</tbody>
</table>

Values within a row and columns 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 tegumental architecture is shown at Figure 1B.

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. (Fig 2).

3.2.2.2. FeO (0.008%)

The result of FeO (0.008%) Conc on worms (A) increase in widening of the ventral sucker (VS); (B) more tegmental bulging and desquamated with loss of spines. (Fig 3).

3.2.2.3. FeO (0.012%)

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 (Fig 4).
the existing problems as many parasitic worms of veterinary importance are able to gradually develop anthelmintic 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 vitatulorum 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 nanoparticles caused widening in ventral sucker and tegmental disruption with roughness, bleeding, 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 Gherbashy 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

