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SHORT COMMUNICATION

Anthelmintic effects of zinc oxide and iron oxide nanoparticles against *Toxocara vitulorum*

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Abstract In the present study, zinc oxide (ZnO) and iron oxide (FeO) nanoparticles were examined for their possible, in vitro anthelmintic effects against *Toxocara vitulorum*. The worms were incubated for 24 h with different concentrations (0.004, 0.008 and 0.012% w/v) of the nanoparticles. The parasite mobility, mortality, superoxide dismutase (SOD) activity, malondialdehyde (MDA) and nitric oxide (NO) level were recorded at different time intervals. The results showed that both of the nanoparticles could significantly decrease worm's mobility, increase mortality rate as well as elevate MDA and NO content as compared to control group in a time- and concentration-dependent manner. SOD activity was elevated with the low concentration of the nanoparticles but it was decreased in higher ones. It can be concluded that ZnO and FeO nanoparticles exert their anthelmintic effects via induction of oxidative/nitrosative stress.

Keywords *Toxocara vitulorum* · Anthelmintic · Zinc oxide · FeOn oxide · Nanoparticle

Introduction

Helminth infections are widespread throughout the world, ranging from tropical, subtropical to temperate climates, affecting both humans as well as livestock animals and cause huge economic losses worldwide [1]. Millions of people from all over the world suffer from diseases such as schistosomiasis, ascariasis and ancylostomiasis, particularly in countries with low public hygiene quality. Such infestations can cause severe conditions, including gastroenteritis, anemia, stunted growth, blindness and lameness [2]. *Toxocara vitulorum*, adults of which are 15–30 cm in length, is a nematode living in small intestines of cattle (*Bos taurus*, *Bos indicus*), Asian water buffalos (*Bubalis bubalis*), and zebu found in tropical and subtropical countries. It is transmitted to calves both transplacentally before birth and via lactation after birth. Infection by water or feed is very rare. In the live stock industry, the parasite causes worldwide economic losses in terms of anorexia and subsequent weight loss, and even death [3, 4]. Until now, several widespread incidences of the parasite infestation have been reported from various regions of the world [3–6]. In the absence of an effective vaccine, the only fruitful choice is chemotherapy to cure and control parasitic disease, but there are several reports of emergence of anthelmintic resistance in parasites [1]. Furthermore, residual of these agents, in domestic animal products (such as milk and meat) can cause critical problems in humans. A survey in South Africa revealed that resistance to anthelmintics is in extremely serious situation and some strains of worms may soon not be controllable by treatment of the current anthelmintics [7]. Therefore, investigation of new agents for treatment of worm's infestation with the ability of high toxicity to parasites and less resistance and residual is the issue of considerable interest for pharmacists and veterinarians.

Recently, many kinds of nanomaterials such as metal oxide nanopowders [8], polymeric metallic nanoparticles [9] and carbon nanotubes [10] have gone under condense

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investigation for their possible pharmacological effects. The similar size of nanoparticles and biomolecules such as proteins and polynucleic acids provide a wide utilization of these materials in biology and medicine [11].

In the recent years, metal oxide nanoparticles have been proposed as antibacterial [12], antiviral [13], antifungal [14], antiprotozoal [15, 16] and anthelmintic [1] agents and several studies have been performed in these fields. ZnO is one of the five zinc compounds that are currently listed as generally recognized as safe by the United State Food and Drug Administration [17]. Since the FeO nanoparticles can be easily sequestered by the spleen and eventually removed by the cells of the phagocyte system, they have been proposed as an excellent agent to drug delivery [18]. In this study, we examined anthelmintic effects of zinc oxide and iron oxide nanoparticles against *T. vitulorum*. Following in vitro exposure of worms with the nanoparticles, parasite mobility and mortality as well as SOD activity, MDA and NO level as markers of oxidative/nitrosative damage were determined.

Materials and methods

Chemicals and reagents

Ultrapure (≥99%), high-quality zinc oxide (20–30 nm) and iron oxide nanoparticles (20–40 nm) were purchased from commercial supplier (Iranian Nanomaterials Pioneers Company, Iran-Mashhad). The nanoparticles were originally synthesized by US Research Nanomaterials, Inc, USA. The certificate of the nanomaterial was provided by the commercial supplier (Figs. 1, 2, 3, 4). All other chemicals and reagents were of the highest commercial grade.

Collection and maintenance of parasites

Adult parasites were collected from the small intestine of freshly slaughtered young cattle at a municipal abattoir of Urmia, Iran. The worms were washed and carefully identified using a light stereomicroscope, then transported in flask containing physiological solution (Figs. 5, 6).

Preparation of ZnO and FeO nanoparticles suspension

Stock solution of the both nanoparticles were prepared by suspending of them at a concentration of 10 mg/ml using sonicator probe (Branson Sonifier, USA) at 30 W for 10 min, while the working solutions of 0.004, 0.008 and 0.012% (w/v) were prepared with Ringer solution [1].

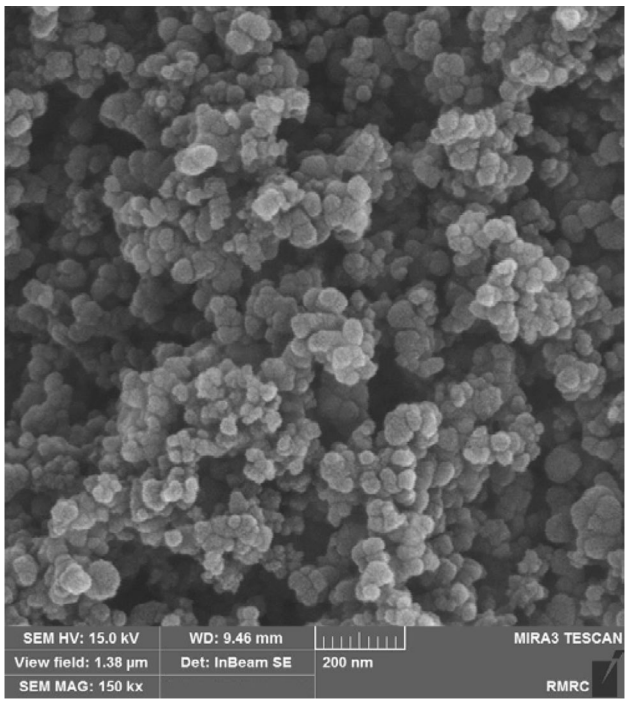

Fig. 1 SEM image of ZnO nanoparticles

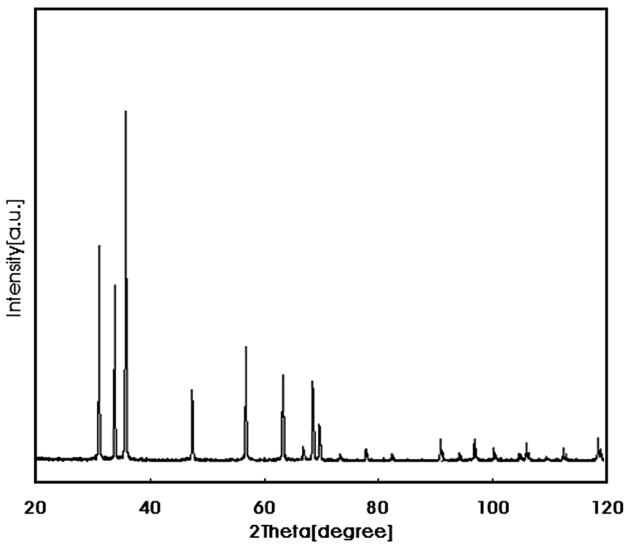

Fig. 2 XRD pattern of ZnO nanoparticles

Incubation of parasites with ZnO and FeO nanoparticles suspensions

Seven conical flasks of 200 ml capacity were labeled with different doses of the nanoparticles and control. Twenty parasites were transported to each flask, containing 100 ml of Ringer solution according to the previously recommended procedure [19, 20] and incubated for 24 h at 37 °C in an atmosphere of 5% CO_2. The worms were monitored at different time intervals for 24 h and the number of dead

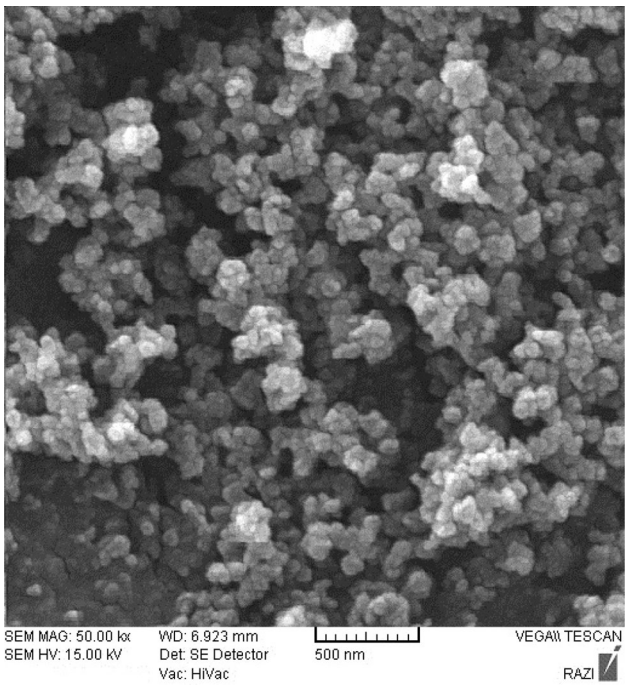

Fig. 3 SEM image of FeO nanoparticles

worms was recorded. After incubation, parasites were washed with PBS several times to remove the adherent particles and used for various studies.

Assessment of parasite motility

The motility of worms was periodically recorded at different time intervals up to the end of the experimental course in various concentrations of the ZnO and FeO nanoparticles along with the control group.

Fig. 5 Two adult *Toxocara vitulorum. Note* large, creamy-white worm with blunt ends

Assessment of biochemical markers

Prior to biochemical analyses, each worm (three for each group) was cut into pieces and mixed with ice-cold 0.86% NaCl. The mixture was homogenized with Ultrasonic processor (Branson Sonifier, USA), and then centrifuged at 4000 rpm and 4 °C for 10 min. The resulting supernatants were used for the determination of superoxide dismutase (SOD), malondialdehyde (MDA) and nitric oxide (NO) levels. SOD activity was assayed using the xanthine–

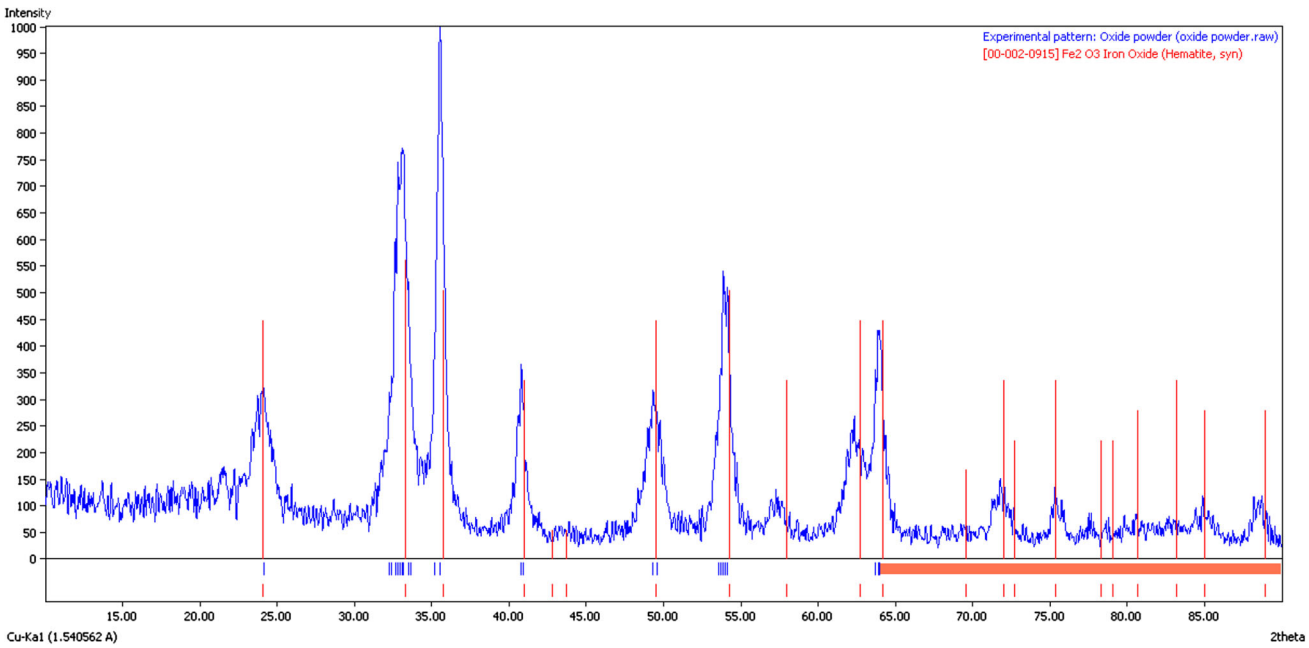

Fig. 4 XRD pattern of FeO nanoparticles

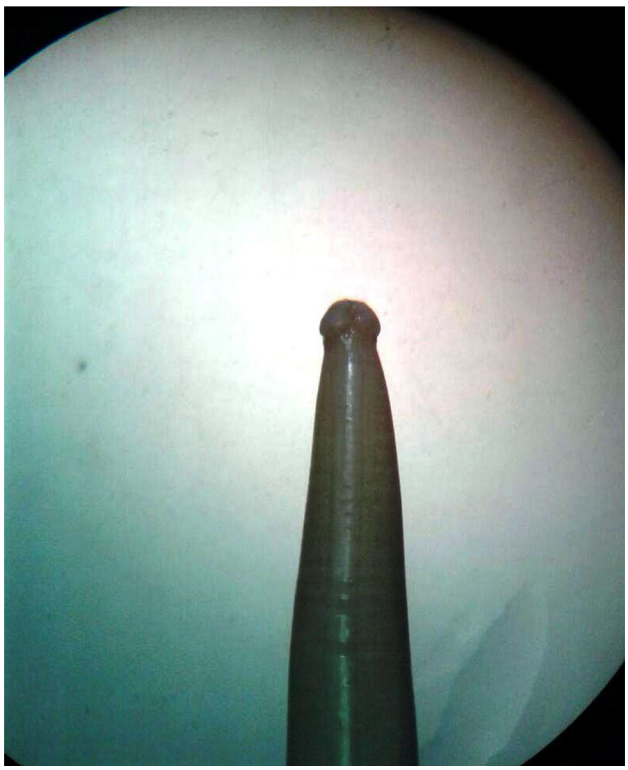

Fig. 6 Wing-like projections (alae) in the anterior end of *Toxocara vitulorum* (magnification ×5)

xanthine oxidase and nitroblue tetrazolium (NBT) system. One unit of SOD was defined as the amount of protein that inhibited the rate of NBT reduction by 50% [21]. Malondialdehyde, a marker of lipid peroxidation, was evaluated as thiobarbituric acid reactive substances (TBARS) by the method of Nair and Turner [22]. The total nitrate/nitrite content of the samples was measured according to the Griess reaction [23]. In Griess reaction nitric oxide rapidly converted into more stable nitrite, which in an acidic environment is converted to HNO_2. In reaction with sulphanilamide, HNO_2 forms a diazonium salt, which reacts with *N*-(1-naphthyl) ethylenediamine. 2HCl to form an azo dye that can be detected by absorbing at 540 nm wavelength. The NO content of the parasite was expressed as nmol per mg of protein in samples.

Statistical analysis

The data were expressed as mean ± standard deviation (SD). Statistical significances of differences among treatments were determined by use of analysis of variance and covariance (ANOVA), followed by Tukey's pair-wise comparisons. A significant difference was presumed at a P value <0.05.

Results

Parasite mobility

The changes in the mobility of worms are represented in Table 1. The movements were carefully checked using a light stereomicroscope. The motility was seriously decreased with the increase in incubation period and concentration of the both nanoparticles. Middle and higher doses of the both nanoparticles reduced the mobility as compared to the respected control. The untreated control worms remained active up to 12 h; however, a slight decrease was observed at the end of incubation period.

Parasite viability

As can be seen from Table 2, both of the nanoparticles showed anthelmintic effects in a time- and concentration-dependent manner. The both have anthelmintic effects in the first 6 h. FeO nanoparticle exerted its maximum effect in 12 h. The data clearly show that within the same dose and time point, FeO nanoparticle exerts greater anthelmintic effects than ZnO.

Table 1 The effect of ZnO and FeO nanoparticles on the mobility of *Toxocara vitulorum*

Groups		Total worm used	Motility rate, time (h)			
			3	6	12	24
ZnO	0.004%	20	++++[a]	++++[a]	+++[b]	+++[b]
	0.008%	20	++++[a]	+++[b]	+++[b]	++[c]
	0.012%	20	+++[b]	++[c]	+[d]	…[e]
Control		20	++++[a]	++++[a]	++++[a]	+++[b]
FeO	0.004%	20	++++[a]	+++[b]	++[c]	++[c]
	0.008%	20	+++[b]	++[c]	++[c]	+[d]
	0.012%	20	++[c]	+[d]	…[e]	…[e]

Values within a row and column carrying different superscript letter (a–e) denote significant differences ($P < 0.05$)

[a] ++++ (High), [b] +++ (Moderate), [c] ++ (Low), [d] + (Very Low/negligible), [e] … (immobile)

Table 2 Effect of various concentrations and incubation time of ZnO and FeO nanoparticles on the worm viability

Groups		Total worm used	Number of worms dead, time (h)			
			3	6	12	24
ZnO	0.004%	20	0^{aI}	$1^{abI\dagger}$	$3^{b\dagger}$	$8^{b\ddagger}$
	0.008%	20	1^{aI}	$3^{bd\dagger}$	$7^{c\ddagger}$	$13^{c\Lambda}$
	0.012%	20	4^{bI}	$9^{c\dagger}$	$17^{d\ddagger}$	$20^{d\Lambda}$
Control		20	0^{aI}	0^{aI}	0^{aI}	2^{aI}
FeO	0.004%	20	0^{aI}	2^{abI}	$5^{b\dagger}$	$11^{c\ddagger}$
	0.008%	20	3^{bI}	5^{dI}	$13^{e\dagger}$	$16^{e\ddagger}$
	0.012%	20	7^{dI}	$15^{e\dagger}$	$20^{f\ddagger}$	$20^{f\ddagger}$

* Values within a column carrying different superscript letter (a–f) denote significant differences ($P < 0.05$)

** Values within a row carrying different superscript letter (I–Λ) denote significant differences ($P < 0.05$)

Table 3 The effect of ZnO and FeO nanoparticles on the (SOD) activity in *Toxocara vitulorum*

Groups		Total worm used	Activity of SOD (U/mg prot)			
			3	6	12	24
ZnO	0.004%	20	9.62 ± 0.11	9.85 ± 0.21	10.3 ± 0.28	11.61 ± 0.11*
	0.008%	20	10.44 ± 0.91	11.32 ± 0.38*	12.57 ± 0.61**	13.21 ± 0.31**
	0.012%	20	11.77 ± 1.15*	12.63 ± 0.10**	8.06 ± 1.54**	7.83 ± 0.47**
Control		20	9.82 ± 0.11	9.71 ± 0.17	10.01 ± 0.2	9.93 ± 0.14
FeO	0.004%	20	9.46 ± 0.13	9.87 ± 0.39	10.62 ± 1.41	11.64 ± 0.09*
	0.008%	20	10.33 ± 0.53	12.28 ± 0.26**	13.95 ± 0.96**	8.71 ± 0.01*
	0.012%	20	11.76 ± 0.31*	8.83 ± 0.05*	7.21 ± 1.16**	8.13 ± 0.81**

Results are expressed as the mean ± SD ($n = 3$). Significantly different from the control: * $P < 0.05$, ** $P < 0.01$

Super oxide dismutase (SOD) activity

SOD activities in the worm after exposure to a series of ZnO and FeO nanoparticle concentrations for 24 h are shown in Table 3. The incubation of worms with the different concentrations of the nanoparticles exerts variable effects on the SOD activity. A slight increase of SOD activities was observed at lowest concentration (0.004% w/v) of both the nanoparticles at the end of the experimental course, while their highest dose severely suppressed enzyme activity.

Malondialdehyde (MDA) levels

The results of malondialdehyde contents are represented in Table 4. No significant change was observed with lowest concentration of ZnO and FeO nanoparticles in the first 6 h. However, the incubation of worms with both of the nanoparticles resulted in elevation of MDA levels in a time- and dose-dependent fashion.

Nitric oxide (NO) levels

The results of measurement of NO for the different groups are depicted in Table 5. As can be seen both of the nanomaterials can induce nitrosative stress in a time- and concentration-dependent fashion.

Discussion

In the current work, the effects of zinc oxide and iron oxide nanoparticles on *T. vitulorum* were assessed. In previous years, several kinds of nanoparticles have been proposed as anti-parasite agent [1, 16, 24]. With respect to increase in resistance to existing anthelmintic, there is an urgent need to investigate novel strategies for control of parasites. Many parasitic helminths of veterinary importance because of their unique genetic features are able to develop gradual anthelmintic resistance; this becoming a major worldwide constrain in livestock production can be considered as a

Table 4 The effect of ZnO and FeO nanoparticles on the MDA levels in *Toxocara vitulorum*

Groups		Total worm used	Malondialdehyde levels (nmol/mg prot)			
			2	6	12	24
ZnO	0.004%	20	1.11 ± 0.06	1.13 ± 0.55	0.98 ± 0.37	1.26 ± 0.01*
	0.008%	20	1.08 ± 0.15	1.29 ± 0.02*	1.54 ± 0.03**	1.85 ± 0.17**
	0.012%	20	1.31 ± 0.05*	1.63 ± 0.06**	1.81 ± 0.10**	2.01 ± 0.06**
Control		20	1.10 ± 0.07	1.08 ± 0.09	0.99 ± 0.05	1.14 ± 0.10
FeO	0.004%	20	1.02 ± 0.03	1.10 ± 0.13	1.28 ± 0.04*	1.67 ± 0.07**
	0.008%	20	1.30 ± 0.17*	1.55 ± 0.11**	1.82 ± 0.20**	2.14 ± 0.16**
	0.012%	20	1.91 ± 0.63*	1.97 ± 0.14**	2.18 ± 0.04**	2.37 ± 0.22**

Results are expressed as the mean ± SD ($n = 3$). Significantly different from the control: * $P < 0.05$, ** $P < 0.01$

Table 5 The effect of ZnO and FeO nanoparticles on the NO levels in *Toxocara vitulorum*

Groups		Total worm used	Nitric Oxide levels (nmol/mg prot)			
			2	6	12	24
ZnO	0.004%	20	25.11 ± 1.09	25.13 ± 1.15	24.98 ± 1.67	29.06 ± 2.01*
	0.008%	20	24.88 ± 2.15	28.49 ± 1.38*	33.04 ± 1.21**	36.85 ± 1.37**
	0.012%	20	30.01 ± 1.15**	35.63 ± 1.16**	39.81 ± 2.18**	46.01 ± 1.86**
Control		20	25.11 ± 0.74	25.08 ± 0.89	24.97 ± 0.95	25.14 ± 1.10
FeO	0.004%	20	25.02 ± 1.03	24.79 ± 0.88	26.58 ± 0.54*	37.67 ± 0.77**
	0.008%	20	26.70 ± 0.47*	31.55 ± 1.09**	35.82 ± 1.20**	38.94 ± 1.16**
	0.012%	20	32.61 ± 2.63**	37.97 ± 1.24**	42.18 ± 2.04**	49.37 ± 1.27**

Results are expressed as the mean ± SD ($n = 3$). Significantly different from the control: * $P < 0.05$, ** $P < 0.01$

serious danger to future production and welfare of grazing animals [25]. The results of the study revealed that both of the nanoparticles are effective on the worm, evidenced by increased mortality rate and decreased mobility of the parasite. The effects were time and concentration dependent. However, it was found that FeO nanoparticles are more effective than ZnO. This can be related to the nature of the nanoparticles. Each nanoparticle is characterized by its own physicochemical features such as, size, specific surface area and surface reactivity. These features can influence nanotoxicological behaviors of nanoparticles [26]. Superoxide dismutase (SOD) plays a critical role in neutralizing of reactive oxygen species (ROS) and is tightly associated with zinc ions. SOD possesses the largest catalytic efficiency of any known enzyme. The enzyme catalyzes the partitioning of the toxic superoxide (O^{2-}) radical with high proficiency [27, 28]. Parasitic nematodes, like all aerobic organisms, require antioxidant enzymes to cope with ROS generated during cellular metabolism. Additionally, they have to protect themselves against ROS produced by the host. Parasitic nematode enzymes that deal with the superoxide anion radical, the superoxide dismutases, have been described in every species examined [29]. Treatment with lower dose (0.004%, w/v) of the both nanoparticles resulted in elevation of SOD activity. These data suggested that ZnO and FeO treatment might result in increase of ROS generation, which could stimulate SOD activity to cope with this increased oxidative stress. However, this protective system appeared to be overwhelmed when the worms were treated with the highest concentration (0.012%, w/v) of the nanoparticles. A significant reduction of the SOD activity was recorded in the *T. vitulorum* following treatment of worms with the highest concentration of the nanoparticles, possibly due to the saturation of the enzyme or consumption of it in partitioning superoxide radicals as a result of overproduction of ROS which renders the detoxification mechanism ineffective. These observations completely match the previous report [1].

Measurement of lipid peroxidation is a golden marker of oxidative damage caused by ROS, and the assessment of MDA is a reliable method to gain such determination [30]. It was observed that the level of MDA was increased time and concentration dependently. This is due to overproduction of free radicals particularly hydroxyl radicals. ROS attacks cell membrane phospholipids and results in peroxidation of them. Such injury to cell membrane finally eventuates in cell death. Nitric oxide is major reactive

nitrogen species in biological systems. NO can react with several oxidative molecules such as molecular oxygen, ROS, transition metals and thiols to yield various reactive nitrogen species and, therefore, induction of nitrosative stress [31]. Oxidative/nitrosative stress can attack biological systems and induce severe damages to biomolecules. Even, it can cause irreversible change and destruction of bio-structures in many of organs. Consistently, in the recent study, it was observed that in vitro administration of plant extract results in irreversible damage to cuticle and hypodermis of *T. vitulorum*, including swelling, distortion and vacuole formation [19]. The similar destructive effect was also observed when ZnO nanoparticle was added to *Gigantocotyle explanatum* media [1]. Oxidative stress not only can cause structural damage, but also can eradicate subcellular architecture such as mitochondria and thereby overwhelms ATP production. This can finally be eventuated in the worm paralysis [32, 33]. Another underlying mechanism for antiparasitic effect of ZnO is induction apoptosis. It is reported that following treatment of *Leishmania major* promastigotes with ZnO nanoparticles, necrotic and apoptotic effects in the parasite were emerged [16]. It is well documented that metal oxide nanoparticles release ROS in huge amount and thereby exert their cytotoxic effects [1, 30]. Taking together it can be concluded that ZnO and FeO nanoparticles exert their anthelmintic effects via induction of oxidative/nitrosative stress.

References

1. Khan, Y.A., Singh, B.R., Ullah, R., Shoeb, M., Naqvi, A.H., Abidi, S.M.A.: Anthelmintic effect of biocompatible zinc oxide nanoparticles (ZnO NPs) on *Gigantocotyle explanatum*, a neglected parasite of Indian Water Buffalo. PLoS ONE **10**(7), e0133086 (2015)
2. Peter, W., Deogracious, O.: The in vitro ascaricidal activity of selected indigenous medicinal plants used in ethno veterinary practices in Uganda. Afr. J. Tradit. Complement. Altern. Med. **3**(2), 94–103 (2006)
3. Aydin, A., Goz, Y., Yuksek, N., Ayaz, E.: Prevalence of *Toxocara vitulorum* in Hakkari eastern region of Turkey. Bull. Vet. Inst. Pulawy **50**(1), 51 (2006)
4. Woodbury, M.R., Copeland, S., Wagner, B., Fernando, C., Hill, J.E., Clemence, C.: *Toxocara vitulorum* in a bison (Bison bison) herd from western Canada. Can. Vet. J. **53**(7), 791 (2012)
5. Davila, G., Irsik, M., Greiner, E.C.: *Toxocara vitulorum* in beef calves in North Central Florida. Vet. Parasitol. **168**(3–4), 261–263 (2010)
6. Mahieu, M., Naves, M.: Incidence of *Toxocara vitulorum* in Creole calves of Guadeloupe. Trop. Anim. Health Prod. **40**(4), 243–248 (2008)
7. Van Wky, J., Stenson, M., Van der Merwe, J., Vorster, R., Viljoen, P.: Anthelmintic resistance in South Africa: surveys indicate an extremely serious situation in sheep and goat farming. Onderstepoort J. Vet. Res. **66**(4), 273 (1999)
8. Dizaj, S.M., Lotfipour, F., Barzegar-Jalali, M., Zarrintan, M.H., Adibkia, K.: Antimicrobial activity of the metals and metal oxide nanoparticles. Mater. Sci. Eng. C Mater. Biol. Appl. **44**, 278–284 (2014)
9. Mody, V.V., Siwale, R., Singh, A., Mody, H.R.: Introduction to metallic nanoparticles. J. Pharm. Bioallied Sci. **2**(4), 282–289 (2010). doi:10.4103/0975-7406.72127
10. Zhang, W., Zhang, Z., Zhang, Y.: The application of carbon nanotubes in target drug delivery systems for cancer therapies. Nanoscale Res. Lett. **6**(1), 555 (2011)
11. De, M., Ghosh, P.S., Rotello, V.M.: Applications of nanoparticles in biology. Adv. Mater. **20**(22), 4225–4241 (2008)
12. Wang, C., Liu, L.-L., Zhang, A.-T., Xie, P., Lu, J.-J., Zou, X.-T.: Antibacterial effects of zinc oxide nanoparticles on *Escherichia coli* K 88. Afr. J. Biotechnol. **11**(44), 10248–10254 (2012)
13. You, J., Zhang, Y., Hu, Z.: Bacteria and bacteriophage inactivation by silver and zinc oxide nanoparticles. Colloids Surf. B **85**(2), 161–167 (2011)
14. Kim, S.W., Jung, J.H., Lamsal, K., Kim, Y.S., Min, J.S., Lee, Y.S.: Antifungal effects of silver nanoparticles (AgNPs) against various plant pathogenic fungi. Mycobiology **40**(1), 53–58 (2012)
15. Baiocco, P., Ilari, A., Ceci, P., Orsini, S., Gramiccia, M., Di Muccio, T., Colotti, G.: Inhibitory effect of silver nanoparticles on trypanothione reductase activity and *Leishmania infantum* proliferation. ACS Med. Chem. Lett. **2**(3), 230–233 (2010)
16. Delavari, M., Dalimi, A., Ghaffarifar, F., Sadraei, J.: In vitro study on cytotoxic effects of ZnO nanoparticles on promastigote and amastigote forms of *Leishmania major*. Iran. J. Parasitol. **9**(1), 6–13 (2014)
17. Premanathan, M., Karthikeyan, K., Jeyasubramanian, K., Manivannan, G.: Selective toxicity of ZnO nanoparticles toward Gram-positive bacteria and cancer cells by apoptosis through lipid peroxidation. Nanomed. Nanotechnol. Biol. Med. **7**(2), 184–192 (2011)
18. Laurent, S., Forge, D., Port, M., Roch, A., Robic, C., Vander Elst, L., Muller, R.N.: Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. Chem. Rev. **108**(6), 2064–2110 (2008)
19. Shalaby, H.A., El-Moghazy, F.M.: In vitro effect of *Nigella sativa* oil on adult *Toxocara vitulorum*. Pak. J. Biol. Sci. PJBS **16**(22), 1557–1562 (2013)
20. Hanser, E., Mehlhorn, H., Hoeben, D., Vlaminck, K.: In vitro studies on the effects of flubendazole against *Toxocara canis* and *Ascaris suum*. Parasitol. Res. **89**(1), 63–74 (2003)
21. Sun, Y., Oberley, L.W., Li, Y.: A simple method for clinical assay of superoxide dismutase. Clin. Chem. **34**(3), 497–500 (1988)
22. Nair, V., Turner, G.: The thiobarbituric acid test for lipid peroxidation: structure of the adduct with malondialdehyde. Lipids **19**(10), 804–805 (1984). doi:10.1007/BF02534475
23. Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S., Tannenbaum, S.R.: Analysis of nitrate, nitrite, and [15 N] nitrate in biological fluids. Anal. Biochem. **126**(1), 131–138 (1982)
24. Sazgarnia, A., Taheri, A.R., Soudmand, S., Parizi, A.J., Rajabi, O., Darbandi, M.S.: Antiparasitic effects of gold nanoparticles

with microwave radiation on promastigotes and amastigotes of *Leishmania major*. Int. J. Hyperth. **29**(1), 79–86 (2013)
25. Shalaby, H.A.: Anthelmintics resistance; how to overcome it? Iran. J. Parasitol. **8**(1), 18–32 (2013)
26. Chang, Y.-N., Zhang, M., Xia, L., Zhang, J., Xing, G.: The toxic effects and mechanisms of CuO and ZnO nanoparticles. Materials **5**(12), 2850 (2012)
27. Tiwari, B.K., Pandey, K.B., Abidi, A.B., Rizvi, S.I.: Markers of oxidative stress during diabetes mellitus. J. Biomark. **2013**, 8 (2013)
28. Kriegel, T., Schellenberger, W.: Biochemie und pathobiochemie. Springer-Lehrbuch, Berlin (2007)
29. Henkle-Dührsen, K., Kampkötter, A.: Antioxidant enzyme families in parasitic nematodes. Mol. Biochem. Parasitol. **114**(2), 129–142 (2001)
30. Nazarizadeh, A., Asri-Rezaie, S.: Comparative study of antidiabetic activity and oxidative stress induced by zinc oxide nanoparticles and zinc sulfate in diabetic rats. AAPS PharmSciTech. **17**(4), 834–843 (2016). doi:10.1208/s12249-015-0405-y
31. Soneja, A., Drews, M., Malinski, T.: Role of nitric oxide, nitroxidative and oxidative stress in wound healing. Pharmacol. Rep. **57**, 108 (2005)
32. Unfried, K., Albrecht, C., Klotz, L.-O., Von Mikecz, A., Grether-Beck, S., Schins, R.P.: Cellular responses to nanoparticles: target structures and mechanisms. Nanotoxicology **1**(1), 52–71 (2007)
33. Wolstenholme, A.J., Fairweather, I., Prichard, R., von Samson-Himmelstjerna, G., Sangster, N.C.: Drug resistance in veterinary helminths. Trends Parasitol. **20**(10), 469–476 (2004)