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Antiparasitic Activity of Chemically Synthesized Magnesium Oxide Nanoparticles against Small Ruminant Haemonchosis

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Abstract

Background: Haemonchosis, a debilitating parasitic disease of small ruminants, is a major threat to food security globally. The development of alternative antiparasitic interventions is an important measure toward reducing the progress of veterinary drugs resistance in food animals. Hence, reducing the risks related to chemical foodborne hazards exposure. This study was designed to investigate the anti-parasitic activity of magnesium oxide nanoparticles (MgO-NPs) against different stages of *Haemonchus contortus*.

Methods: Preparation of magnesium oxide nanoparticles was conducted by sol-gel method at room temperature. For anti-parasitic activity of nanoparticles and commercially available anthelmintics, the adult worms and eggs (collected from gravid female worms) were subjected to adult motility assay and egg hatch assay, respectively. The relative efficacy of both nanoparticles and anthelmintics was classified by identifying the lethal dose LC50.

Results: Wormicidal effects of MgO-NPs were found to depend on both the concentration and the amount of time, they were exposed to. The lower concentrations of the nanoparticle (1 to 5 µg/mL) have not shown any mortality during the first four hours of the incubation. However, the higher concentrations of the nanoparticle (10, 15, 20, and 25 µg/mL) harmed the parasite during this time. The highest dose (25 µg/mL) was capable of killing almost all the worms during the first 16 hours. The highest concentration of nanoparticle induced 90±2.89% egg hatch inhibition and 91±3.3% adulticidal activity for egg hatch assay and adult motility assay, respectively.

Conclusion: Magnesium oxide nanoparticles have shown potent anti-parasitic properties. The study may provide potential alternative anthelmintic to control gastrointestinal parasites particularly haemonchosis in the scenario of emerging anthelmintic resistance.



Introduction

Parasitism represents one of the key factors threatening global food security and safety. Gastrointestinal (GI) parasitic nematodes are a major health concern for small ruminants in developing countries. Among various GI parasites, *Haemonchus* (H.) *contortus*, is a primary, economically authorized and very pathogenic abomasal nematode parasite, and is the main culprit associated with limited performance and production of small ruminants in the subtropical and tropical regions [1,2]. The disease caused by this nasty parasite is hemonchosis. The parasite affects an exceedingly large number of small ruminants ensuing in considerable economic losses in terms of declined meat, milk and wool production, reduced body weight, and cost of anthelmintic drugs [3]. Adult H. *contortus* invade abomasal walls, attach themselves, and feed on blood. Muscle thickening, cell hyperplasia, necrosis of mucosal layers, and fibrosis of glandular and inflammatory cells are the chief histopathological changes associated with haemonchosis [4].

In developing countries like Pakistan, the economic losses related to H. *contortus* in small ruminants have been reported more than 8800.09 million rupees in different areas due to condemnation of abomasum. It is also reported that about 29% reduction and 27% reduction in milk yield and weight occurred, respectively due to haemonchosis so, the annual loss in Pakistani rupees is 13406.39 million and 40 million, respectively [5]. The control of GI parasites is mainly centered on the use of anthelmintic drugs (benzimidazoles, levamisoles, and macrocyclic lactones) and plant extracts [6,7]. However, the control of these parasites with current anthelmintic drugs is becoming exceedingly difficult due to the emerging issue of drug resistance [7]. Developing drug resistance is a threat to global food safety and security. Resistance of sheep nematodes to levamisole, oxfendazole, and ivermectin has been reported in some studies as 59%, 88%, and 41%, respectively. Therefore, it necessitates the development of an alternate but eco-friendly and cost-effective product by using nanotechnology.

Nanoparticles (NPs) are widely used for the control of parasites. They cause the death of parasites as they can easily cross the cell membrane due to their smaller size and produce reactive oxygen which results in reactivity and death [8,9]. Magnesium oxide (MgO-NPs) are diversely used in vitro and in vivo biological applications. It may have good anthelmintic properties, as the antiprotozoal activity of MgO-NPs has been reported in Saudi Arabia [10]. But no studies have been reported to determine the in vitro efficacy of chemically synthesized MgO-NPs against nematode parasites, particularly H. *contortus*. Thus, the present study was designed to analyze the comparative anthelmintic

efficacy of chemically synthesized MgO-NPs and economically available anthelmintics.

Methods

Collection and processing of samples

Abomasum of small ruminants, immediately after slaughtering regardless of sex and age of animals were collected and transported to the Molecular Parasitology Laboratory, Department of Parasitology, Faculty of Veterinary Science, University of Agriculture, Faisalabad. The adult female and male worms were collected from the abomasum. The collected worms were washed and finally placed in a petri dish comprising phosphate buffer saline (1x PBS) at pH 7.4 and macerated to obtain eggs which were repeatedly washed with distilled water.

Egg hatch assay

The efficacy of nanoparticles and anthelmintics was determined through egg hatch assay [11]. For collection of eggs, gravid female worms were reduced by using mortar and pestle. The suspension was made using PBS and constrained through a tea strainer to remove all kinds of debris from the suspension. In the end, 200 eggs/mL were maintained through the McMaster technique [12]. Among three experimental groups formed, group one received chemically synthesized MgO-NPs while group two was treated with commercially available anthelmintic (Albendazole) as the positive control. The third group having egg solution and diluent was considered as the negative control. In the assay, approximately 200 eggs in 0.5mL of suspension were placed in each test tube (5mL). Serial dilutions of MgO-NPs consisting of (0.00001, 0.0001, 0.001, 0.01, 0.1 and 1µg/mL) serve as experimental group while Albendazole dissolved in DMSO at different concentrations 0.03125, 0.0625, 0.125, 0.250 and 0.5 µg/mL as positive control group. Test tubes were covered and left at room temperature for 48 hours. The hatching of eggs ceased by adding 2 drops of Lugol's iodine to each tube. Total eggs (dead and embryonated) and the number of hatched larvae were counted in every tube under the microscope at 40X magnification.

Worm in-vitro assay

Worm in-vitro assay was performed to check the efficacy of nanoparticles and anthelmintic as described by [13]. 10 adult worms were selected and kept in each tube containing chemically synthesized MgO-NPs at different concentrations. Worms were placed in PBS and then transferred in petri plate, separately. Each petri plate was provided with serial dilutions of MgO-NPs consisting of 1, 5, 10, 15, 20, and 25 µg/mL. Albendazole dissolved in DMSO at different concentrations of 16, 32, 64, 128, and 256 µg/mL for positive control while phosphate buffer saline as a negative control group. The

anthelmintic efficacy of MgO-NPs was assessed by restricted movement of the worm. After 24 hours duration, the test solution was washed thoroughly, and the worm was suspended in PBS for 30 minutes to recover the remaining movement of the worm. The number of actively moving worms was recorded under a microscope. The mortality percentage was calculated by using the following formulae.

Statistical Analysis

Probit analysis was performed to calculate LC50 values for egg hatch assay and worm in-vitro assay.

Results

For in vitro worm assay, the variation in adult worm motility was depicted both in the experimental and positive control groups. As the incubation duration and concentrations were increased, there was a significant decrease in the amount of movement presented by the worms. With an increase in the concentration of the NPs, the motility was significantly reduced and the mortality percentage increased. In comparison, the mortality was higher even at lower doses of the nanoparticles while the mortality was lower even at much higher concentrations of Albendazole. Figure 1 and 2 show probability plots for antiparasitic effects of MgO-NPs and albendazole on adult worm mortality, respectively. Table 1 and 2 depict probit analysis of MgO-NPs and albendazole to estimate LC50 value by maximum likelihood analysis.

The results of the egg hatch assay were recorded after 48 hours under the microscope at 40X. The hatched larvae and unhatched eggs were counted. The MgO-NPs had a greater impact on inhibition of egg hatching as compared to albendazole, which is less effective even at higher doses. The data unambiguously demonstrate that MgO-NPs deliver stronger antiparasitic effects at the same dose and time point. Figures 3 and 4 indicate probability plots for antiparasitic effects of MgO-NPs and albendazole on the hatching of eggs, respectively. Table 3 and 4 show probit analysis of MgO-NPs and albendazole to estimate LC50 value by maximum likelihood analysis.

Discussion

Haemonchus contortus is one of the leading causes of reduced animal production due to high morbidity and mortality rates [14]. Infection with this nasty parasite causes a decrease in protein absorption, incorrect protein metabolism, and mineral consumption [15]. Although the mortality percentage may not be high during infection, its indirect consequences on animal output are more significant [16]. Decreases in growth, diarrhea, anorexia, loss of appetite, and even anaemia are the indirect losses caused by *H. contortus* [17].

Moreover, inappropriate and misuse of anthelmintics, made the parasite capable of developing resistance against different anthelmintic groups [18,19,20,21]. Hence, to combat the increasing anthelmintic resistance there is a need to adapt alternate ways. In this regard, nanoparticles are one of the emerging alternatives against resistant parasites. Their small size, extraordinary surface reactivity, and wide range of biological applications make them a suitable candidate for parasite control [22]. They can permeate membranes, and produce reactive oxygen species (ROS), which cause intense reactivity and ultimately results in the death of pathogenic agents [23]. The anthelmintic potential of nanoparticles is currently being continuously assessed for the management of parasitic illnesses [9].

The current study investigates the ovicidal and adulticidal efficacy of chemically produced MgO-NPs against *H. contortus* using adult mortality and egg hatch assays. MgO-NPs with dimensions of 6 nm in diameter, and 461 m² in the surface area produced potent antiparasitic effects. After 48 hours, MgO-NPs had egg hatch inhibition of 94.66%, with the highest concentration. Albendazole, a synthetic substance, caused 65.33 % egg hatch inhibition at a dosage of 0.5 g/mL. The MgO-NPs treatment's LC50 value for the egg hatch assay was calculated to be 0.0003984g/mL. According to the study, MgO-NPs were particularly successful at preventing egg hatching at very low concentrations, and nano-sized MgO-NPs must have caused hindrance in molting and other physiological functions necessary for *H. contortus* eggs to hatch [24]. Therefore, it is evident from the literature review that the findings of present study demonstrated the NPs to be more effective [10,25,26,27]. The greatest concentration of MgO-NPs (25ug/mL) caused 96% of adult mortality in the current study. The LC50 value for the MgO-NPs treatment was 11.80238 g/mL. In the current investigation, morphological changes i.e. coil formation by the worms to prevent stress, caused by the test compounds have been utilized as prospective anthelmintic agents. Remarkable anthelmintic activity of MgO-NPs has been found during the current study. The mode of action of MgO-NPs is still debatable.

Transcuticular diffusion has been demonstrated to be a typical route of entry for non-electrolytic compounds in worms in earlier research [28]. Additionally, research has indicated that, as compared to oral intake, this is the preferred method for the absorption of powerful broad-spectrum anthelmintics [29]. Lipophilic anthelmintics, such as albendazole, are better able to penetrate the helminths' outer surface than hydrophilic substances [30].

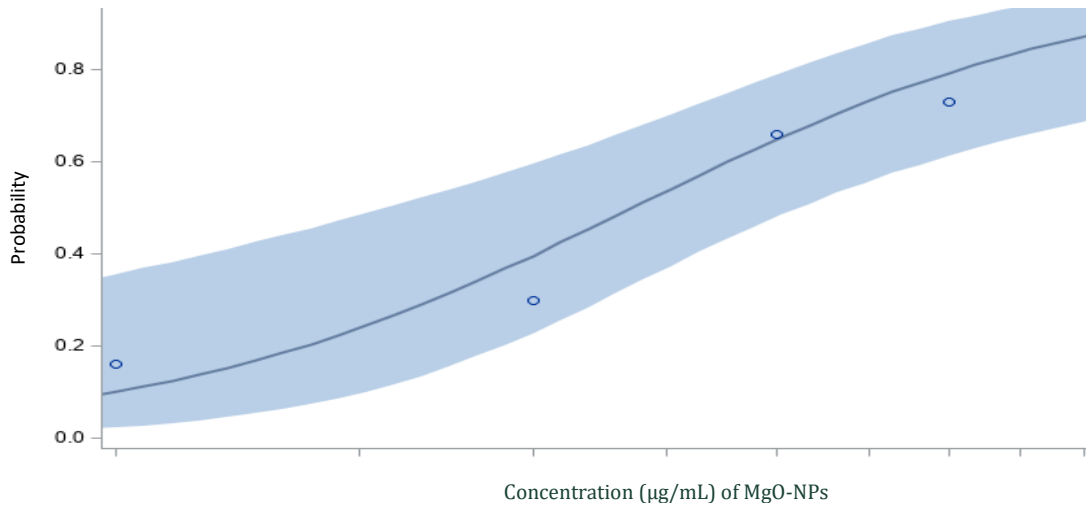


Figure 1: Probability plot for in vitro worm assay using MgO-NPs (lognormal -95% CI)

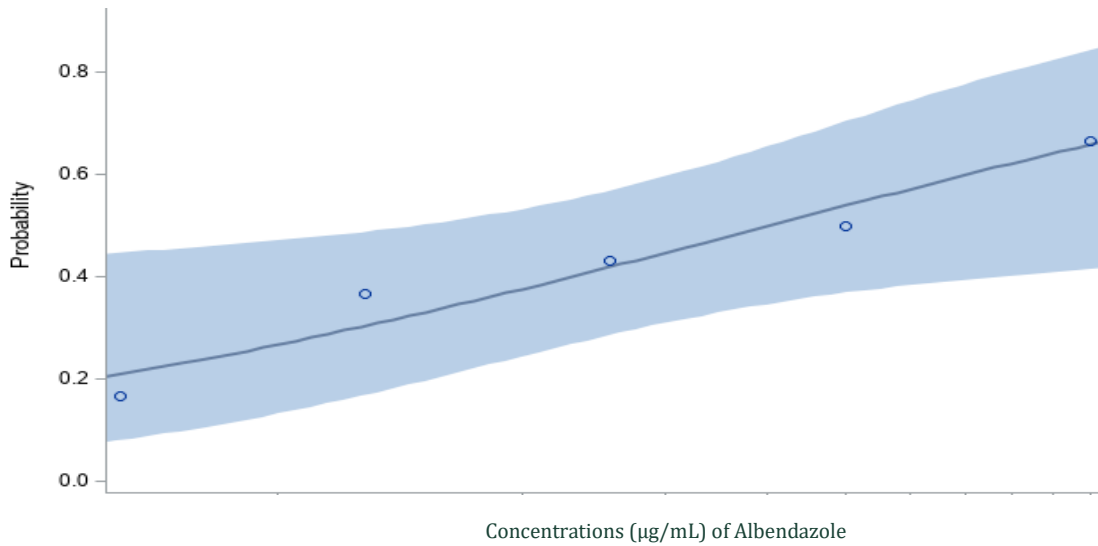


Figure 2: Probability plot for in vitro worm assay using Albendazole (lognormal -95% CI)

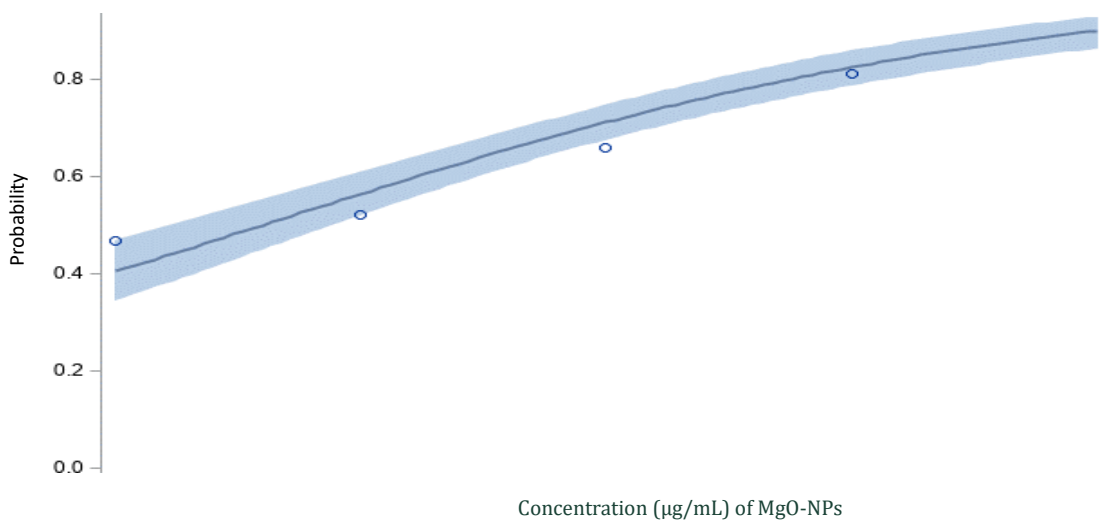


Figure 3: Probability plot for egg hatch assay using MgO-NPs (lognormal -95% CI)

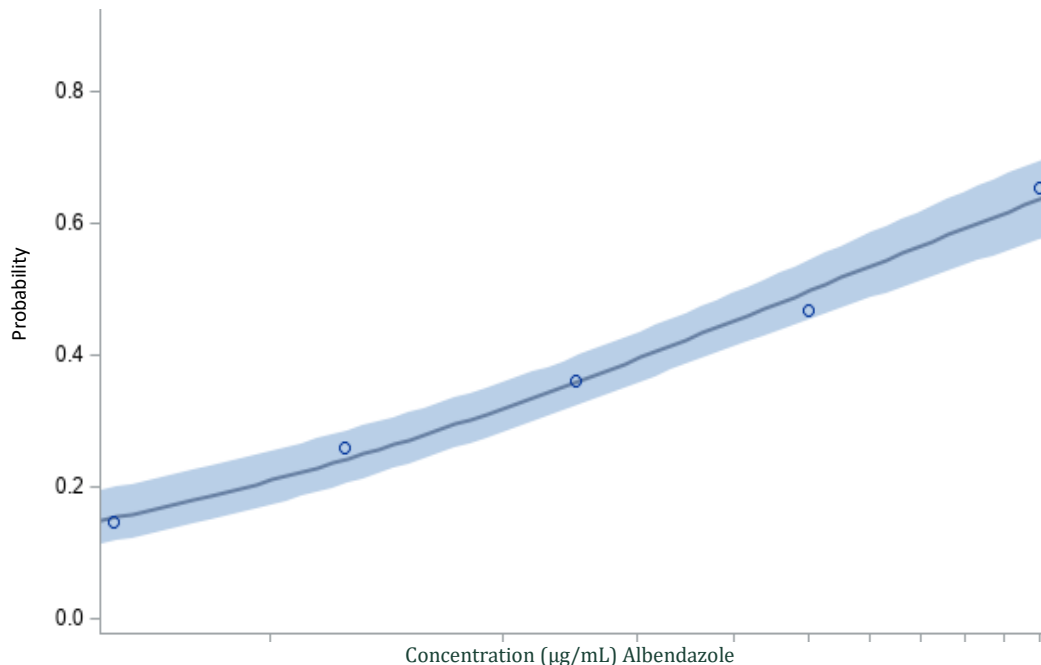


Figure 4: Probability plot for egg hatch assay using Albendazole (lognormal -95% CI)

Analysis of Maximum Likelihood Parameter Estimates							
Parameter	DF	Estimate	Standard Error	95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept	1	-6.3042	±1.9626	-10.1509	-2.4576	10.32	0.0013
Log10(Dose)	1	5.8810	±1.7123	2.5249	9.2371	11.80	0.0006

Table 1: Probity analysis of MgO-NPs for worm in vitro assay to estimate LC50 value by maximum likelihood analysis

Analysis of Maximum Likelihood Parameter Estimates							
Parameter	DF	Estimate	Standard Error	95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept	1	-3.3395	±1.4188	-6.1202	-0.5588	5.54	0.0186
Log10(Dose)	1	1.6667	±0.7514	0.1940	3.1393	4.92	0.0265

Table 2: Probity analysis of Albendazole for worm in vitro assay to estimate LC50 value by maximum likelihood analysis

Analysis of Maximum Likelihood Parameter Estimates							
Parameter	DF	Estimate	Standard Error	95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept	1	2.1920	±0.1812	1.8370	2.5471	146.42	<.0001
Log10(Dose)	1	0.6448	±0.0658	0.5159	0.7737	96.10	<.0001

Table 3: Probity analysis of MgO-NPs for egg hatch assay to estimate LC50 value by maximum likelihood analysis

Analysis of Maximum Likelihood Parameter Estimates							
Parameter	DF	Estimate	Standard Error	95% Confidence Limits		Chi-Square	Pr > ChiSq
Intercept	1	1.1215	0.1847	0.7594	1.4835	36.86	<.0001
Log10(Dose)	1	1.8814	0.2018	1.4860	2.2769	86.95	<.0001

Table 4: Probity analysis of Albendazole to estimate LC50 value by maximum likelihood analysis

However, the extremely small size of the nanoparticles, i.e., 15–25 nm, which likely allowed for transcuticular absorption of MgO-NPs by the parasite, may have contributed to a higher mortality rate of adult parasites following MgO-NPs treatment in the current study as compared to treatment with the anthelmintics. The anthelmintic effects of MgO-NPs against the GI nematode, *H. contortus*, have been studied for the first time in this study. However, various scientists have studied the antibacterial properties of MgO-NPs and reported that the antibacterial efficacy was size dependent, with the smallest sizes having an impact on both Gram-positive and -negative bacteria while bigger sizes exclusively have an impact on Gram-negative ones [31,32,33,34,35,36,37,38]. Additionally, investigations have shown that MgO-NPs with a particle size of less than 50 nm partially suppressed bacterial growth while having a substantial bactericidal impact at that size [39,24,20,21].

The production of superoxide anions O₂ on the MgO surface is one explanation for the strong anthelmintic effects of increasing MgO NP concentrations. The concentration of MgO and OH⁻ on the target's surface is increased as a result of MgO being hydrated and generating Mg (OH)₂, which covers the surface. As a result, the target is more effectively destroyed as the O₂ concentration rises [40-42]. Although NPs size, dosages, and the characteristics of the microbe, including cell wall structure and cellular enzymes, play an essential role as an antibacterial agent, other parameters such as NPs structure, surface qualities, and the temperature of synthesis may also be contributing to this role. The effects of MgO nanoparticles on *Leishmania* parasites, which exhibited the best antiparasitic activity (anti-leishmanial) among all evaluated NPs, were in line with the findings of the current study on treating worms and eggs [24]. Comparing MgO-NPs to other anthelmintics previously utilized against *H. contortus*, treated worms in the current study showed that they were more effective with MgO-NPs. According to the study's findings, *H. contortus* is very resistant to anthelmintics. It has been determined that among gastrointestinal nematodes, *H. contortus* is the most prevalent species affecting the production of small ruminants. It was discovered that nanoparticles were more efficient than commercially available anthelmintics.

The resistance to commercially available chemical anthelmintics is growing daily as a result of the widespread usage of chemical substances. The findings of this study showed that MgO-NPs are more effective against *H. contortus*. However, haemonchosis and other worm-related disorders can be controlled with the help of nanoparticle-based anthelmintics, which are also effective against *H. contortus*. Animals that have

already developed resistance to chemical anthelmintics, i.e. sheep and goats are unable to eradicate GI nematodes even after treatment with the required amount of anthelmintics, benefit more from nanoparticle-based anthelmintics. Commercially available anthelmintics have less efficacy even at higher concentrations as compared to nanoparticles. These agents are needed in higher doses to have desired effects. Nanoparticles have good anthelmintic activity even at very low concentrations. The result of the study shows that the anthelmintic effect of nanoparticles is in a dose-dependent manner for both parameters, which suggests the use of these NPs for control of GI nematodes.

Competing interest

The author declares that there is no conflict of interest regarding the publication of this paper.

Authors' Contribution

Muhammad Imran planned, executed, and conducted the research in the laboratory. Abdullah F Alsayeqh helped in troubleshooting, statistical analysis, and drafted the manuscript.

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