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Antiprotozoal activity of magnesium oxide (MgO) nanoparticles against *Cyclospora cayetanensis* oocysts

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ABSTRACT

Outbreaks of *Cyclospora cayetanensis* infection have been linked to consumption of food and water contaminated by oocysts that can survive both physical and chemical disinfectants. Magnesium oxide (MgO) nanoparticles (NPs) can be potentially used in food as bactericides. In this study, *C. cayetanensis* pre- and post-sporulated oocysts were exposed to MgO NPs with different doses ranging from 1.25–25 mg/ml. With comparison to control, the antiprotozoal activity of MgO NPs was evaluated by identifying the median effective concentration dose (EC_{50}), lethal concentration dose (LC_{90}), microscopically changes on treated oocysts and rates of sporulation. Among pre- and post-sporulated oocysts, MgO NPs ≥ EC_{50} was observed after 24 h at concentrations 10 and 12.5 mg/ml, respectively, while ≥ LC_{90} was observed after 24 h, 48 h and 72 h at concentrations 15, 12.5 and 10 mg/ml, respectively. MgO NPs treated oocysts showed abnormal morphological changes such as an increase in size, wall injury, deposition of vacuolated homogenous particles in the cytoplasm, evacuation of oocyst's contents, and collapse. Sporocysts of treated oocysts were noticed to be peripherally shifted. Sporulation failure of treated oocysts achieved ≥90% after 24 h and 72 h of incubation with 15 and 12.5 mg/ml, respectively, while it was 10.1% among non-treated. All the differences were statistically significant. Our results demonstrated that MgO NPs has a significant anti-*Cyclospora* effect on both unsporulated and sporulated oocysts, especially considering that it could be biologically synthesized, that way it can be used safely as a preventive agent in food and water disinfectant treatment.

1. Introduction

Apicomplexan *Cyclospora cayetanensis* is an obligate intracellular protozoon that leads to a significant morbidity and mortality in both immunocompromised and immunocompetent infected patients [1]. Worldwide *C. cayetanensis* is reported as the main pathogen of many epidemic and endemic diarrheal diseases with an infection rate that reaches 41.6%, particularly in developing countries [2]. Contaminated water, food, and soil by sporulated oocysts are the main sources of infection transmission [3]. The host specificity of *C. cayetanensis* seems to be exclusively for humans. No other animal species have been identified with this parasite [4]. Usually, infected persons excrete unsporulated oocysts that require about two weeks under ideal conditions to sporulate [5]. Oocyst of *C. cayetanensis* is spherical, 8–10 μm in diameter and in case of unsporulation status it contains a sporont that is full of a cluster of membrane-bound refractile globules [6]. On sporulation the entire sporont divides into two ovoid structures which are called sporocysts, each one is 4 × 6.3 μm and contains two sporozoites and each sporozoite measures 1.2 × 9.0 μm [5]. The amount of time required for oocyst to sporulate in nature is unknown but under laboratory conditions, sporulation occurs after 5–11 days incubation in either distilled water or 2.5% potassium dichromate at temperatures of 25 to 32 °C [4,5]. Laboratory diagnosis of cyclosporiasis by microscopy is the common method but the molecular tool has the potential to be more sensitive in spite of being unable to differentiate live from dead oocysts [7]. Due to the several outbreaks caused by cyclosporiasis and its relation to certain local or imported food and contaminated water supply, the development of new antiprotozoal agents to eradicate the

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resistance infective forms are urgent [2].

Antimicrobial agents particularly the inorganic material are increasingly being used for decontamination [8]. The key advantages of these inorganic antimicrobials, as compared to their organic counterparts, are the safety and stability effects [9]. Recently, clusters of atoms called nanoparticles (NPs) that have a size range of 1–100 nm, are gaining impetus in the present century as they capture defined optical and mechanical properties [10]. These nanoscale materials have emerged up as novel therapeutic agents owing to their high surface area to volume ratio compared with macro-sized particles [11]. Furthermore, the technology of nano is capable of providing miscellaneous novel applications that range from innovative fabric compounds, food processing, and agricultural production to sophisticated medicinal techniques [12]. Nanosize metals can be more effective in comparison with bulk metal [13]. Different types of nanomaterials metals like copper, zinc, titanium [14], magnesium, gold [15], alginate [16] and silver [10] were developed and produce different degrees of antimicrobial effects. In waterborne pathogenic protozoa *Cryptosporidium parvum* NPs such as silver and copper oxides were used as preventive tools that significantly decreased oocysts viability and infectivity [17]. MgO has some advantages that prior it to other metals such as non-mutagenic effect, good stability and it is a normal component of the human body [18]. Several studies revealed that MgO is able to deactivate both Gram-negative and Gram-positive bacteria through several mechanisms of deactivation [19]. Moreover, bacterial cell wall distortion and damage occurred with MgO NPs treatments lead to leakage of intracellular contents and eventually bacterial death [20]. Recently, biologically synthesized of MgO NPs from *Clitoria ternatea* by calcination enhanced its antioxidant activity [21]. Until now MgO NPs activity was only assessed against one parasite, *Leishmania major*, and noticed a strong anti-leishmanial activity against promastigote when compared to all tested NPs such as silver, gold, titanium dioxide and zinc oxide [22].

Therefore the aim of this study was to identify the antiprotozoal activity of MgO NPs against *C. cayetanensis* oocysts treated pre and post sporulation.

2. Material and methods

2.1. C. cayetanensis oocysts source

C. cayetanensis oocysts were obtained from five stool samples of heavily infected cyclosporiasis patients (the number of oocysts was ≥4oocysts per low power field (X10) according to Dixon et al. [23]. All of them were attending gastroenterology clinic, Suez Canal University Hospital, Ismailia, Egypt. Verbal consents were obtained from the patients and all of the procedures were conducted according to the ethical standard approved by the institutional human ethics committee, Faculty of Medicine, Suez Canal University, Egypt. Each positive sample was kept separately and involved in the experiment at once.

2.2. Isolation and purification of C. cayetanensis oocysts

C. cayetanensis oocysts were isolated from 5 mg or 5 ml of feces through suspension in 10 ml phosphate buffered saline (PBS, pH 7.4) and filtrated through four layers of gauze to remove the coarse material. The filtrate was centrifuged at 800*g* for 5 min. After elimination of the supernatant, a modified Sheather's sugar solution (without phenol) having a specific gravity of 1.27 was added to 2 ml of the sediment and then centrifuged at 600*g* for 20 min. The float fluid was collected and examined for the presence of *C. cayetanensis* oocysts by both of wet mount and Kinyon staining methods. DNAs were extracted from positive concentrated samples to confirm the diagnosis of cyclosporiasis with polymerase chain reaction (PCR). At the same time oocysts of *C. cayetanensis* were stored in 2.5% potassium dichromate solution (1: 4) at 4 °C for until confirmed by PCR [24]. All oocysts involved in the MgO NPs experiments study were not more than one week old. *Cyclospora* oocysts obtained from each stool sample were used separately per each MgO NPs dose. In each time, assays were conducted in triplicate per each sample. Whereas each MgO NPs concentration dose was tested by three stool (oocysts) samples.

2.3. Molecular confirmation of C. cayetanensis oocysts

Unsporulated *Cyclospora* oocysts were washed three times with phosphate-buffered saline (PBS, pH 7.4) and centrifuged at 4300*g* for 5 min to remove all the potassium dichromate [24]. Then 200 µl from the sediment were used in DNA extraction using the QIAamp DNA micro kit (Qiagen, Mississauga, Canada) according to manufacturer instructions. Controls positive and negative were obtained from our previous samples [25]. According to Relman et al. [26], PCR amplification was performed using 18S rRNA as the target gene in a nested PCR assay generated a 294 bp amplicon.

2.4. MgO NPs preparation

Large-scale one-dimensional MgO nanowires with diameters of 6 nm and lengths of 10 µm and a surface area about 461 m2/g, were successfully synthesized by a new facile and simple reaction via a microwave hydrothermal approach at a low-temperature growth of 180 °C for 30 min [27]. The structure of synthesized MgO nanowires was investigated by means of X-ray diffraction, Fourier transformation infrared spectroscopy, field emission scanning electron microscopy (SEM), transmission electron microscopy (TEM), selected area electron diffraction and energy dispersive x-ray. Two MgO NPs experiments were performed. The first was testing the effect of MgO NPs on pre -sporulated oocyst. The second was testing the effect of MgO NPs on sporulated oocysts. In the current experiments, non-treated oocysts were used as a control negative per each experiment. Whereas, the positive control oocysts were obtained from heating non-treated oocysts in water at 70 °C for ≥15 min [4]. The lethal concentration dose (LD_{90}) was defined as the lowest concentrations of MgO lethal to 90% of oocysts after exposure and effective concentration (EC_{50}) was defined as the median effective concentrations of MgO lethal to 50% oocyst after exposures. Also, the treated and non-treated oocysts were examined by SEM and TEM to identify any changes on oocyst's wall, morphology, and ultrastructure according to Speer et al. [28].

0.5. 2Effect of MgO NPs on unsporulated oocyst.

C. cayetanensis oocysts were washed four times with PBS (pH 7.4) by centrifugation at 4300*g* for 5 min to remove potassium dichromate then the oocysts in the remained pellet were enumerated using a Neubauer hemocytometer (W. Schreck, Hofheim/TS, Germany) from the stock preparation. The oocysts were diluted with PBS to prepare inoculums of 50×10^8 oocysts /ml. Then, six concentrations of MgO NPs from the lowest concentration to highest (1.25, 2.5, 5, 10, 12.5 and 15 mg/ml) were added and the final volume was adjusted to be 50×10^4 oocysts /ml. The mixture was incubated at 37 °C for required test time interval to assess the mortality (destructive effect) rate (MR), EC_{50} and LD_{90} that were identified after daily examination of the sediments. Then, the pellets were re-suspended in 1 ml of PBS (pH 7.4). The tubes were centrifuged again at 1120*g* for 10 min, and the supernatant volume was reduced to 0.2 ml. Then, the sediments were used in counting the appearing intact treated oocysts compared to the original count and non-