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Original article

# Assessment of the oxidative status and goblet cell response during eimeriosis and after treatment of mice with magnesium oxide nanoparticles

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ABSTRACT

Magnesium nanoparticles have been the focus of study over the past few years because of its functionality in the body. Assessment of the impact of magnesium oxide nanoparticles (MgNPs) on eimeriosis has yet to be conducted. The goal of this study was to see how MgNPs affected the parasite *Eimeria papillata* infected jejunum. To induce eimeriosis, mice were infected with sporulated oocysts. For treatment, 5 mg / Kg MgNPs was used for 5 consecutive days. The infection reduced the number of intestinal goblet cells and their associated genes *MUC2* and *MUC4*, as well as increasing oxidative damage in the jejunum. MgNPs significantly reduced the oocyst production in the feces by about 77 %. After treatment, the number of goblet cells per villus increased from 4.17% to 7.40.6%. Moreover, the MgNPs were able to upregulate the expression of *MUC2* and *MUC4*-mRNA. MgNPs significantly increased the activity of catalase and superoxide dismutase, as well as the extent of glutathione, by day 5 after infection with the parasite. On contrary, MgNPs decreased the level of malondialdehyde and nitric oxide. The findings suggested that MgNPs could be an effective anti-eimeriosis agent due to their anti-eimerial and anti-oxidant roles, as well as the regulatory effect on the goblet cell mucin genes in the jejunum of mice.


## 1. Introduction

Coccidiosis continues as one of the most destructive diseases of the animals worldwide (Mehlhorn 2014). Parasitism is related to the *Eimeria* genus which causes numerous problems in the poultry industry (See in Lawal et al. 2016).

Researchers are seeking to find environmentally ecofriendly agents to fight the parasite. Because of their safety and stability, antimicrobial chemicals, particularly inorganic products, are extensively employed for therapeutic purposes (Makhluf et al., 2005). Recently, specific forms of nanomaterials have been produced including metals such as silver, gold, copper, zinc and magnesium, creating various degrees of antimicrobial activity (Hussein et al., 2018). Moreover, Alkhudhayri et al. (2020) reported the antioxidant and the antiparasitic effect of nanoselenium.

Magnesium oxide nanoparticles (MgNPs) received extensive interest of researchers due to the ease of synthesis, chemical stability and extensive applications in different fields. In medicine, MgNPs are used in therapy for the treatment of pathological diseases such as bacterial infection and for the delivery of drugs (Alexa et al., 2012) due to nontoxic, environmentally friendly often with minimal side effects (Alexa et al., 2012). At the parasitological level, the anti-Cyclospora effect of MgNPs was evaluated (Hussin et al., 2018). In addition, the effect of MgNPs on Leishmania major was assessed and a significant anti-leishmanial activity against promastigote was investigated (Jebali and Kazemi 2013). Our previous research discovered that another metal oxide nanoparticle, zinc oxide nanoparticles, exhibits anti-emerial function (Dkhil et al., 2015). Our aim was to assess the role of MgNPs during

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oxidative stress induced by *E. papillata* and to study the response of goblet cells and their related genes during infection and after administration of MgNPs.

## 2. Material and methods

### 2.1. Synthesis and characterization of MgNPs

MgNP synthesis was performed (see in Wahab et al., 2007) using a soft chemical and hydrolytic method and MgNPs were characterized by Scanning Elecetron Microscopy (JEOL Ltd., Tokyo, Japan) (Nguyen and Harbison 2017).

### 2.2. Experimental infection

Twenty-one male mice (C57BL/6) were used (25–30 g). Animals were kept at standard conditions and fed a normal diet. Oocysts count in feces of infected animals were done as mentioned in Dkhil et al. (2015).

To prepare the oocysts for model mice infection, the produced feces were incubated in 2.5 % potassium dichromate for sporulation.

Mice were allocated into 3 groups containing seven animals in each group. The control group, administered orally with distilled water. Groups two and three were inoculated orally, with 1,000 *E. papillata* sporulated oocysts suspended in distilled water. Group 3 was also given an oral dose of MgNPs (5 mg/kg) for 5 days (Jahangiri et al., 2013).

### 2.3. Goblet cells response

Sections for the identification of goblet cells in jejenum were cut, processed, and then stained with Alcian blue (Dkhil et al., 2015). This is after being processed to obtain paraffin sections.

### 2.4. Oxidative status in jejunum

Pieces of mouse jejunum were weighed and then homogenized in tris(hydroxymethyl)aminomethane (Tris)-HCl and sucrose. Then, the separated supernatant (only 10%) was used for the different biochemical assays.

Using the method of Ellman (1959), the concentration of glutathione (GSH) was calculated in the jejunum homogenate. For malondialdehyde (MDA), Ohkawa et al. (1979) method was performed. The nitric oxide (NO) level was determined according to Berkels et al. (2004). The enzyme activities of catalase (CAT) and superoxide dismutase (SOD) were assayed as mentioned in Aebi (1984) and Nishikimi et al. (1972), respectively.

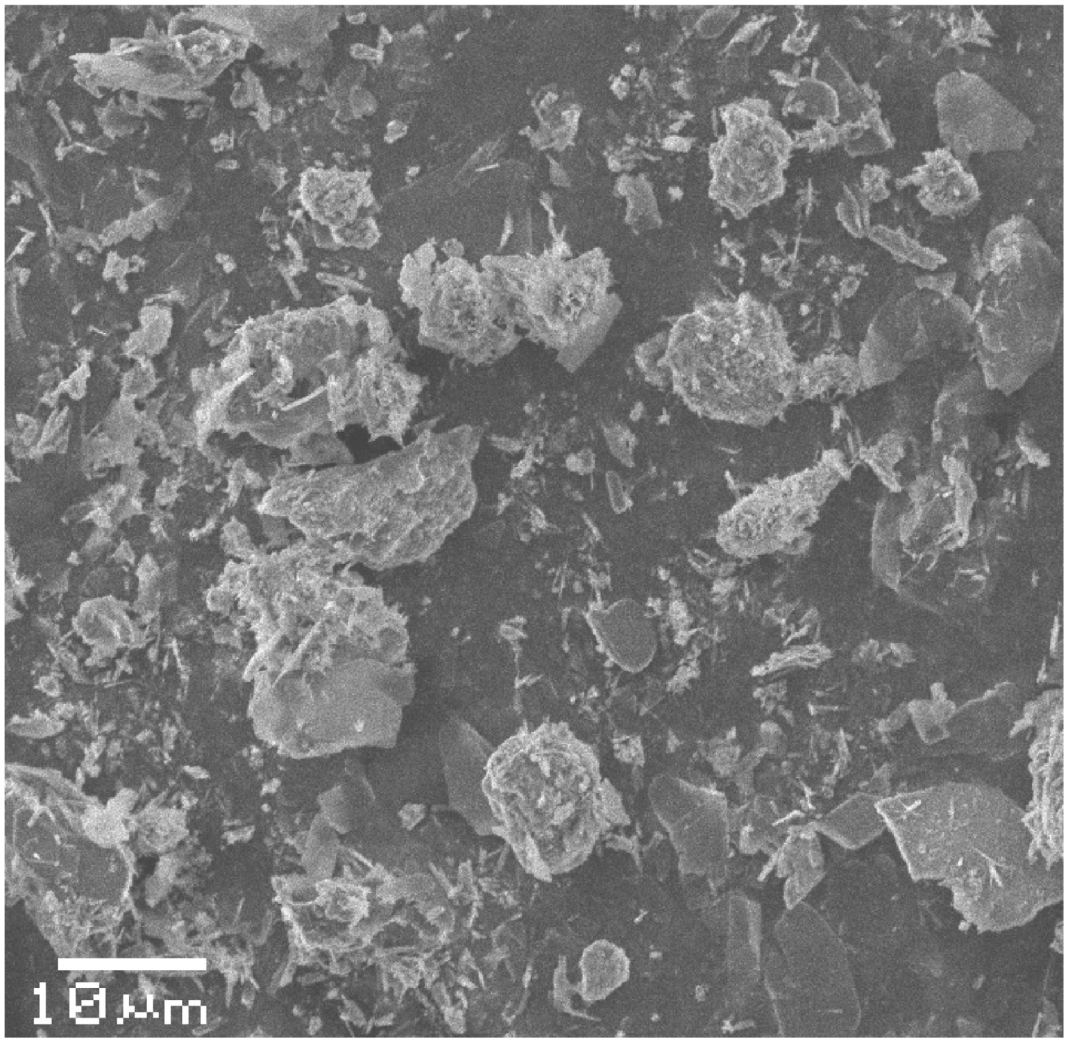


**Fig. 1.** TEM image of MgNPs nanoparticles. The particle size was around 10–15 nm, and the shape was spherical.

### 2.5. Mucin gene expression

Total RNA was prepared from the jejunum using Trizol (Invitrogen) and then transformed to cDNA using a reverse transcription kit (Qiagen, Hilden, Germany). As mentioned in Alkhudhayri et al. (2020), the quantitative real-time PCR (qRT-PCR) was conducted. Both *MUC2* and *MUC4* mRNA expression were determined using the commercially obtained Qiagen qRT-PCR primers. The levels of mRNA had been normalized to 18S rRNA.

### 2.6. Statistical analysis

One-way ANOVA was used and the significance between groups was considered where * and # are significance against control and infected, respectively at $p < 0.01$.

## 3. Results

The shape as well as the size of MgNPs were determined where the TEM figure clearly illustrated the average size between 10 and 15 nm and spherical in shape (Fig. 1).

Treatment of mice with MgNPs caused a significant decrease in oocysts of the infected mice. This reduction of oocysts reached about 3.5 fold compared to the infected animals (Fig. 2).

The infection caused a marked decrease in goblet cells (Fig. 3) but after treatment of mice with MgNPs, the goblet cells appeared significantly more than the infected group (Fig. 4).

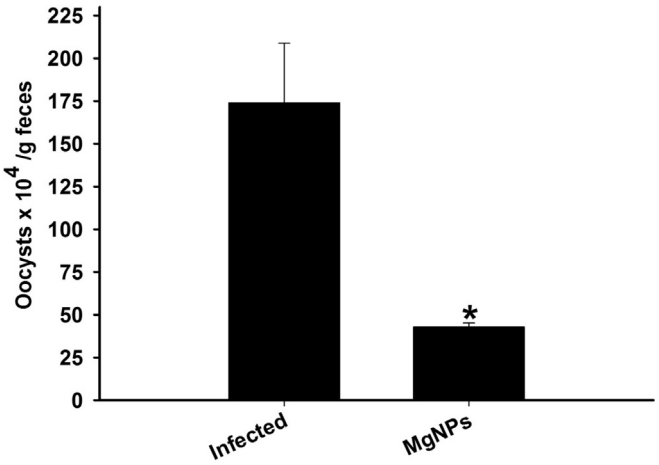


**Fig. 2.** Effect of MgNPs nanoparticles on the oocysts expelled in mice faeces due to *E. papillata* infection.

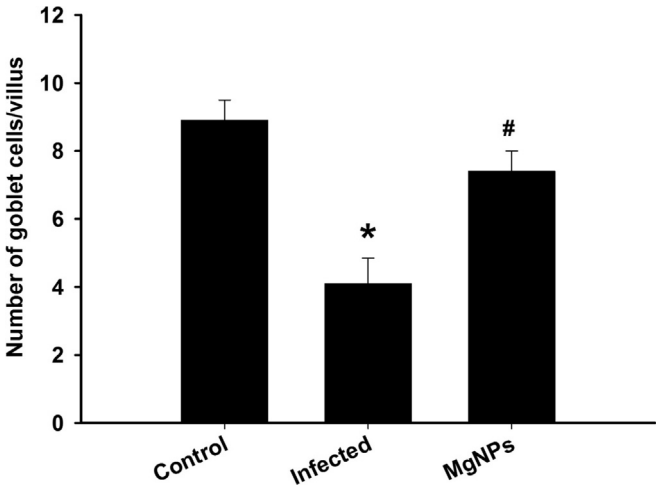


**Fig. 4.** MgNPs nanoparticles effect on the number of infected mice goblet cells.

The two mucin genes *MUC2* and *MUC4* expression were decreased due to infection. However, MgNPs were able to significantly upregulate these genes (Fig. 5).

The infection caused unbalance in the oxidative status in the jejunum. This was demonstrated by the estimation of the level of GSH, MDA and NO and the activity of CAT and SOD (Table 1).

There was a noticeable reduction ($P < 0.01$) in GSH concentration as well as in the activity of CAT and SOD in the infected group compared to the control one. However, MAD and NO levels were increased due the infection (Table 1). MgNPs caused clear amelioration in the concentration of GSH, NO and CAT while MDA and SOD change were non-significant compared to the infected group (Table 1).

## 4. Discussion

Mg is needed for a wide range of physiological and biochemical processes like RNA synthesis, DNA or protein, and membrane stability (Schweigel and Martens 2000). It also plays an important role in monitoring membrane channels (Rasgado-Flores et al., 1994) as well as in the coupling of excitation contraction in the skeletal muscle (Lamb and Stephenson, 1994). In addition, MgO nanoparticles have been used in applications for antacids, detoxifiers, biomolecular identification and diagnostics (Bertinetti et al., 2009; Singh et al., 2020).

In this study, MgNPs was able to decrease the oocyst output in mice faeces. Russell and Sinden (1981) reported that, Mg ions have inducted an inhibitory activity on *Eimeria magna*'s sporozoite

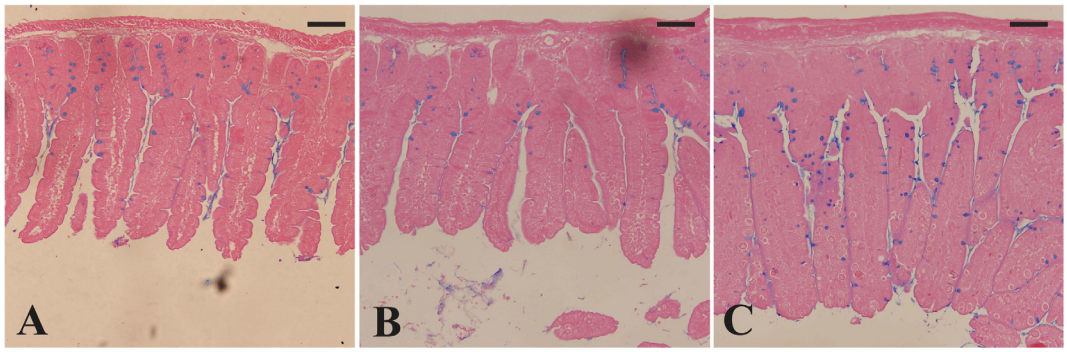


**Fig. 3.** Alcian blue stained jejunum sections. (A), Non-infected jejunum with higher number of goblet cells then *E. papillata* infected one (B). (C) Infected-MgNPs treated jejunum with increased goblet cells than the infected jejunum.

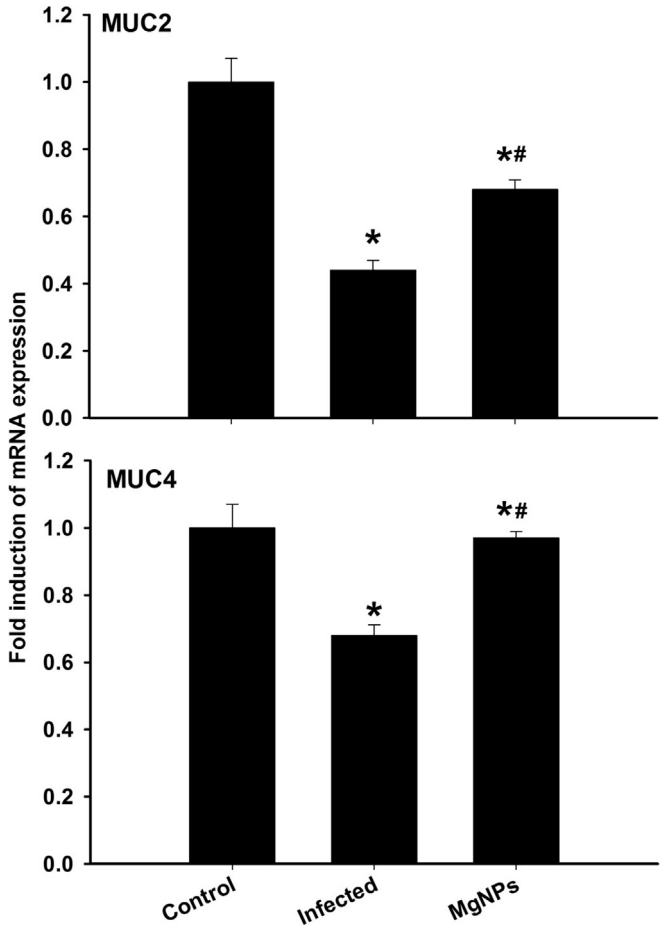


**Fig. 5.** MgNPs nanoparticles effect on the mucine genes, *MUC2* and *MUC4* expression.

**Table 1**
Effect of MgNPs on the concentration of oxidative stress markers in jejunum of mice infected with *E. papillata*.

| Group | GSH (mg/mg protein) | MDA (nmol/mg protein) | NO (μmol/mg) | CAT (U/mg) | SOD (U/mg) |
|---|---|---|---|---|---|
| **Control** | 0.57 ± 0.05 | 72.4 ± 6 | 1.3 ± 0.1 | 63.5 ± 6 | 18.9 ± 1.3 |
| **Infected** | 0.39 ± 0.03 * | 99.4 ± 7 * | 1.8 ± 0.18 * | 42.1 ± 5 * | 13 ± 1.5 * |
| **MgNPs** | 0.45 ± 0.02 *,# | 88.8 ± 7 * | 1.42 ± 0.1 # | 56.7 ± 5 # | 14.2 ± 1.5 * |

Data presented as mean ± SD. * and # are significance against control and infected, respectively at $p < 0.01$.

motility. This positive impact was probably due to the microfilamantous system disruption (Lin et al. 1978).

The mucus secreted by goblet cells facilitates for the removal of intestinal content and represents the first line of protection against the damage caused by ingested food, microbes, and parasites (Parmar et al 2021).

The stages of *E. papillata* parasite occur in the jejunal crypt site (Cheng, 1974). The reduction in number of goblet cells may be due to parasite damage to the stem cell population in the crypts. Evidently, goblet cells are formed by mitosis from stem cells at the jejunal crypt (Cheng, 1974). Alterations in the number of goblet cell affect the infected host's susceptibility to opportunistic pathogens' ability to interact with or penetrate the target epithelium.

Our results indicated that the expression of mucin genes was downregulated during infection and after treatment of mice with MgNPs, there was an upregulation in goblet cell *MUC2* and *MUC4* genes. Shan et al. (2014) reported that the presence of magnesium is important for the mucin biological function in normal and disease state.

We concentrated extensively in this study on the oxidant / antioxidant status during eimeriosis. It was clear that MgNPs during infection with *E. papillata* exhibit anti-coccidal and anti-oxidant activity. Esch and Petersen (2013) clarified that, the antioxidant defense system's unbalance due to infection with *Eimeria* contributes to adverse cellular effects (Esch and Petersen 2013).

*E. papillata* increases the activity of catalase (Metwaly et al., 2015; Thagfan et al., 2021) through converting hydrogen peroxide to water and oxygen then $H_20_2$ levels are reduced (George 1947). However, after Eimeria infection, the level of the biomarker malondialdehyde rises (Dkhil 2013) and the application of MgNPs ameliorate the oxidative injury caused by the parasite.

MgNPs acted as excellent antioxidants, reducing lipid peroxidation and increasing SOD activity due to increased $O_2$ scavenging after infected mice were treated with MgNPs.

Our results collectively show that MgNPs possess anticoccidial and antioxidant properties and also could regulate the mucin genes in mice jejunum infected with *E. papillate*. The mechanism of MgNPs action during eimeriosis should be studied in future. Also, the behavioral and metabolic studies should be considered.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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