Effect of ZnO nanoparticles in treatment of broilers with Eimeria tenella. experimentally infected.

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Research Article

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Abstract

Coccidiosis is one of the common poultry diseases in Iraq and the world, which causes great economic losses. Therefore, this study aimed to use a new treatment represented by nanoparticles of zinc oxide and compare it with the drug (Amprolium).

The Oocysts of *Eimeria tenella* were isolated from the feces of the broilers received at the Veterinary hospital in Diwaniyah Province and initially diagnosed by compound light microscopy, Then it was confirmed molecularly by PCR technique using the internal transcribed space ITS1 gene with a molecular weight of 409bp.

The experiment was carried out on 120 birds of broilers, as the animals were divided into 6 groups with 20 birds per group. The first group (G1) was left as a negative control (uninfected), the second group (G2) was inoculated with 50,000 mature Oocysts and left as a positive control group, the third group (G3) was incubated with 50,000 Oocysts + Amprolium (anticoccidial), the fourth, fifth and sixth groups (G4, G5, G6) were also dosed with 50,000 Oocysts followed by an oral dose of nanoparticles of zinc oxide at a concentration of 20, 40, 60 mg/kg for each of fourth, fifth and sixth groups respectively.

The current results showed that the broilers in G2 suffered from severe disease symptoms and high mortality occurred in the first week of infection, amounting to %10 compared to the anticoccidial (Amprolium group and the groups treated with nanoconcentrations 20, 40, 60 mg/kg respectively. G6 which was dosed with a concentration of 60 mg/kg, showed a significant decrease in the number of Oocysts excreted compared with the positive control group and the anticoccidial group, in addition to the appearance of mild symptoms and a mortality rate of %0.8, While there was a decrease in the number of Oocysts excreted during the second week in the anticoccidial group and the treated groups with the three nanoconcentrations with a decrease in the mortality rates of birds. The number of Oocysts excreted in the litter significantly decreased in the treated group with a concentration of 60 mg/kg compared to all other groups, and all birds of this group recovered during the second week of infection.

Introduction

Nanomaterials play an important role in a wide range of biology such as medicine, industry and environmental sustainability (Crane & Scott, 2012; Bai *et al.*, 2009). In the folding field many experiments have been conducted that include the use of nanoparticles for the purpose of activating the performance of medicinal drugs in the form of systems that deliver and transporting drugs as drug-carrying nanoparticles for the purpose of treating cancer cells (Korbekandi & Iravani, 2012) as nanomaterials can kill 650 cells (Sungkaworn *et al.*, 2007).

Due to the developments that appear on microbes and their resistance to drugs many researchers and scientists have resorted to searching for new solutions represented by the use of nanotechnology and one of the most important of these nanoparticles is zinc oxide nanoparticles which have the advantage of being an antimicrobial, growth enhancer, a nutritional supplement for humans and animals, modified
Immunostimulant, a catalyst for phytochemical reactions and low toxicity, what distinguishes these nanoparticles is their large surface area compared to their small size and thus they are able to deal with biological reactions within cells (Swain et al., 2016; Al-Zahrani et al., 2018).

occidiosis is one of the important parasitic diseases and one of the difficult problems facing poultry farming in Iraq and the world, as it causes great economic losses despite the development of many means to limit its spread such as good management, methods of improving breeding, preventing chicks from eating mature Oocysts through the use of the floor mesh in the breeding and use of sterilizers, but most of them have not been adequately proven to be effective (Hausermann, 1999; Shirzad et al., 2011).

Materials And Methods

1. Diagnosis of *E. tenella* Oocysts

Oocysts were obtained from infected broiler specimens imported to the Veterinary hospital in Al-Diwaniyah Province, where the stool was placed in a potassium dichromate solution%2.5, then the flotation process was performed by adding saturated saline (saturated sodium chloride) to flotation the Oocysts.

A haemocytometer was used to calculate the number of Oocysts used to infect a broiler with *E. tenella* parasite based on (Jorgensen et al., 1997; Al-Attar, 1981), Oocysts were counted in the boxes designated for counting white blood cells, then the total number of Oocysts was divided by 8 to obtain the average per square, then multiplied by 10,000 to produce the number of Oocysts in 1ml.

\[
\text{account of Oocysts in 1 ml} = \frac{\text{account of Oocysts in 8}}{8} \times 10000
\]

The diagnosis of Oocysts of the parasite was confirmed using PCR technique, where a pair of primers for the gene Internal Transcribed Space ITS1 with a molecular weight of 409bp was designed based on Hamidinejat et al.(2010).

**ITS1:F-** AGCAGGTAGTCGTCGGTGTT, **ITS1:R-** AGCAAAGTTCCAAGCAGCAT

DNA was extracted using a stool Genomic DNA extraction kit equipped from the Korean company Bioneer. The concentration and purity of the extracted DNA was measured by a Nanodropspectrophotometer. After completing the PCR master mix using the AccuPower® PreMix kit and according to the instructions attached to the kit, the tubes containing PCR components and other components were closed and mixed with it, vortex for five seconds and then tubes were transferred to a PCR Thermocycler.

2. Characteristics of ZnO nanoparticles

This compound was purchased in the form of ready-made oxide from one of the US companies approved by Sky Spring Nanoparticles Inc. In the form of a white to light yellow powder with a purity of %99.8 and a
size of 10-30 nm.

The nanocomposite (ZnONPs) was prepared in the form of a stock solution, where 50 gm of ZnONPs were dissolved in 100ml of water distilled and sterilized with an autoclave, then the solution was mixed using an ultrasonic homogenizer for 15 minutes, then the concentrations (20,40,60) mg/kg were prepared and kept in Refrigerator until used in the experiment.

3. Experiment animals.

120 birds from briolers, one day old were obtained from the Diwaniyah hatchery(Turkish Rose strain) and all the chicks were reared on the mattress from one day old until the end of the trial period .And all groups were subjected to the same environmental conditions of feeding and lighting and followed the preventive and health program of the World Health Organization WHO in breeding briolers.

4. Infection to animals.

After making sure that there were no clinical signs during the first three weeks (21 days) of the chicks' life by examining the stool daily before starting the experiment to ensure that there were no Oocysts for E.tenella, the experimental animals were divided into six groups with 20 birds per group. Experimental infection with E.tenella was induced at 21 days old and dosed orally using a purpose-built stomach tube.

G1 was left as a negative control group (without infection) and was given distilled water, G2 dosed 50,000 mature Oocysts and left as a positive control( group infected), G3 was dosed with 50,000 Oocysts and given anticoccidial ,G4,G5 and G6 were dosed with 50,000 mature Oocysts and also an oral dose of ZnONPs at a concentrations of (20, 40,60)mg/kg respectively.

Results

To confirm the diagnosis of the Oocysts of E. Tenalla (Fig.1) which was used in the experimental infection, DNA was extracted from stool samples taken from infected broilers imported to the veterinary hospital and then subjected to PCR technology to confirm the diagnosis of the parasite(Fig.2).The results of amplification of the gene IST-1 appeared in 100 stool samples positive out of 150, with a total percentage of %66.6.

The results of (Fig.3) which show the number of Oocysts raised during the first week of the experimental infection, indicate that the negative control group which was dosed with distilled water only were healthy birds and did not show any pathological symptoms and the feces of this group were free from Oocysts, while the positive control group showed that dosed with 50,000 Oocysts severe disease symptoms of lethargy of chicks ,severe bloody diarrhea ,reduced consumption of feed, and a %10 mortality in this group and Oocysts were observed in the feces of these birds, which reached 6080 Oocysts.

In the G3 which was dosed with the anticoccidial (Amprolium) a decrease in the number of Oocysts excreted in the stool, the appearance of mild symptoms in most of the birds of this group, and a mortality
rate of %1.6, as for the G4 which dosed 50,000 Oocysts plus a ZnONPS solution at a concentration of 20 mg/kg, it did not lead to a decrease in the number of Oocysts excreted in the feces, as it reached 3000 Oocysts, with symptoms similar to the positive control group, in addition to a %7.5 death rate. Whereas, the G5 which dosed 50,000 Oocysts followed by a dose of ZnONPS solution at a concentration of 40 mg/kg showed a slight decrease in the number of Oocysts, with moderate disease symptoms and death in birds of this group by %3.

The G6 which dosed 50,000 Oocysts followed by a dose of ZnONPS solution with a concentration of 60 mg/kg, showed a significant decrease in the number of Oocysts excreted when compared with the positive control group and the anti-coccidiosis group, in addition to the emergence of mild symptoms and a mortality rate of %0.8. The results of the statistical analysis revealed the presence of significant difference between the positive control group and the other groups at the probability level $P \leq 0.05$.

The results in (Fig.4) also indicated a decrease in the number of Oocysts raised during the second week in the anticoccidial group and the groups treated with the three nanoconcentrations, with a decrease in the mortality rates of birds. The concentration of 60 mg/kg recorded a clear superiority and a significant decrease of $P \leq 0.05$ in the number of Oocysts excreted in the litter compared to all other groups, and all birds of this group recovered during the second week of infection.

**Discussion**

Recent research in the poultry industry indicates that adding zinc in the diet plays an important role in stimulating the immune response, growth of bones and muscles and the growth of feathers and skin, in addition to increasing the bioavailable sources of zinc such as Zin complex to broiler diet leads to weight gain and improves the efficiency of food conversion, it is also necessary for the activity of about 250-300 enzymes and participates in many enzymatic functions and metabolism in the body (Kidd *et al.*, 1996; Ahmadi *et al.*, 2013; Prasad *et al.*, 2002), as well as increases IgY production, total number of lymphocytes, and improves immune responses in general. (Abedini *et al.*, 2018; Azza *et al.*, 2020).

It is worth noting that it is preferable not to use high concentrations of ZnOPS because it is a toxic element when it exceeds the required level (Wang, 2007).

The dosing of the positive control group with Oocyst to *E. tenella* led to the emergence of clinical symptoms represented by general weakness of the bird, decline in growth, and the abstention of some infected birds from eating feed and water, as well as severe bloody diarrhea as a result of the spores penetration of the epithelial tissue of the cecum and the growth and development of the parasite to the stage of the schizogony phase inside the cells epithelial of the cecum, which leads to damage to the capillary blood vessels and occurrence of hemorrhage, and this is consistent with what were mentioned (Gawad *et al.*, 2012; Morris *et al.*, 2007; Iacob& Duma, 2009; Györke *et al.*, 2013).

It was noticed through the results of the current study that the broilers which was infected with the Oocysts of *E. tenella* and which was subsequently dosed at a concentration of 20 mg/ kg of ZnONPs
solution during the first week did not achieve a positive result in reducing the number of Oocysts excreted in the stool. Most of the infected birds suffered from symptoms similar to those of the positive control group, in addition to the occurrence of death in birds of this group. When comparing this group with the anticoccidial group, we find that the anti-coccidiosis reduced the number of Oocysts excreted by acting as an antibiotic that killed the developmental stages in the parasite's life cycle.

As for the concentration of 40 mg/kg of ZnONPs solution, it led to a slight reduction in the number of Oocysts excreted in the stool. Birds of this group showed satisfactory symptoms, but they were lighter than the positive control group, but the concentration of 60 mg/kg recorded a significant decrease in the number of Oocysts thrown into the litter in addition to a mortality rate of %0.8.

The results also showed a clear decrease in the number of Oocysts excreted during the second week in the anticoccidial group and in the treated groups with the three nanoconcentrations with a decrease in the mortality of birds. The concentration of 60 mg/kg recorded a clear superiority and a significant decrease of $P \leq 0.05$ in the number of Oocysts excreted in the litter compared to all other groups, and all birds of this group recovered during the second week of infection.

The reason for the decrease in the Oocysts in the broiler at a concentration of 60 mg/kg of ZnONPs during the second week can be explained by the fact that ZNOPs weakened the development of this parasite before the inactive Oocysts were formed and this is agreement with the findings of Dkhil et al., 2015, as it was shown that ZNOPs It shows anti-activity for *E. papillata* activity and significantly decreased Oocyst excretion in the stool of infected mice.

Or perhaps the ability of ZnONPs to cross the gastrointestinal tract and then be further distributed in the blood and in the target organs, which in turn leads to increased immune responses and resistance to infection. This is in agreement with Yusof et al., 2019.

In addition, there is research indicated that ZnONPs act as liver protective agents in rabbits infected with coccidiosis (Khaled et al., 2018). Recently, ZnONPs and L-carnitine have been shown to have anti-neurological bilharaziasis effects in infected mice (Bauomy, 2020), as well as Bafundo et al. (1984) clearly demonstrated that zinc utilization use is diminished by *Eimeria acervulina* infection.

**Conclusions**

Through the current experience, we have come to prove the role of nanoparticles of zinc oxide in reducing the incidence of coccidiosis, especially at a concentration of 60 mg/kg.

**Declarations**

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Conflict of interest statement

The authors announce that we have no conflict of interest.

Author contribution statement

A.A. Sadiya & A.A.Saad contributed to collecting Oocysts samples, orally dosing birds and statistically analyzing data. A.H.Khadeeja contributed to implementation of PCR. All authors discussed the results, commented on the manuscript and gave final approval of the final version to be submitted.

Ethical approval

All procedures conducted in animals studies were in accordance with the ethical standards of the institution at which the studies were conducted. and the current study was approved by the Committee of Department of biology, Faculty of Education, University of AL-Qadisiyah.

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**Figures**

![Figure 1](image-url)
A: unsporulated Oocyst, B: sporulated Oocyst

**Figure 2**

PCR products of the ITS1 gene of E. tenella after electrophoresis, M-ladder, samples (1-14) represent the positive results for the amplified gene 409 bp.

**Figure 3**

The effect of ZnONPS on the number of Oocysts excreted in broilers feces and the mortality rates in each group in the first week.
Figure 4

Effect of ZnONPS on the number of cysts excreted in broilers feces and mortality rates in each group in the second week.