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Original Article

The Therapeutic Efficacy of Zinc Oxide Nanoparticles on Acute Toxoplasmosis in BALB/c Mice

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Abstract

Background: *Toxoplasma gondii* infects nearly one-third of the world's population. Due to the significant side effects of current treatment options, identifying safe and effective therapies seems crucial. Nanoparticles (NPs) are new promising compounds in treating pathogenic organisms. Currently, no research has investigated the effects of zinc oxide NPs (ZnO-NPs) on *Toxoplasma* parasite. We aimed to investigate the therapeutic efficacy of ZnO-NPs against tachyzoite forms of *T. gondii*, RH strain in BALB/c mice.

Methods: In an experiment with 35 female BALB/c mice infected with *T. gondii* tachyzoites, colloidal ZnO-NPs at concentrations of 10, 20, and 50 ppm, as well as a 50 ppm ZnO solution and a control group, were orally administered four hours after inoculation and continued daily until the mices' death. Survival rates were calculated and tachyzoite counts were evaluated in the peritoneal fluids of infected mice.

Results: The administration of ZnO-NPs resulted in the reduction of tachyzoite counts in infected mice compared to both the ZnO-treated and control group ($P<0.001$). Intervention with ZnO-NPs significantly increased the survival time compared to the control group (6.2±0.28 days, P-value <0.05), additionally, the highest dose of ZnO-NPs (50 ppm) showed the highest mice survival time (8.7±0.42 days).

Conclusion: ZnO-NPs were effective in decreasing the number of tachyzoites and increasing mice survival time in vivo. Moreover, there were no significant differences in survival time between the untreated control group and the group treated with zinc oxide, suggesting that, bulk ZnO is not significantly effective in comparison with ZnO-NPs.

Introduction

Toxoplasma gondii is an obligate intracellular protozoan parasite that belongs to the phylum Apicomplexa and causes toxoplasmosis (1). *T. gondii* infects approximately one-third of the global population (2). Cats are definitive and all warm-blooded animals and humans are intermediate hosts (3).

Humans are primarily infected by consuming raw or undercooked meat, contaminated vegetables or water, or through vertical transmission. There is also a rare possibility of transmission through blood and organ transplantation (3). Following ingestion, bradyzoites and sporozoites within cysts and oocysts, are released and invade the epithelial cells of the intestine, where they differentiate into tachyzoites. Through the blood circulation or lymphatic system, tachyzoites can be disseminated to distant organs leading to acute and chronic toxoplasmosis (4).

Immunocompetent patients with *T. gondii* infection are often asymptomatic (5), but immunocompromised patients may experience severe symptoms, including life-threatening conditions (5, 6). Pregnant women with toxoplasmosis are at risk of complications such as miscarriage, blindness, and mental disorders in their fetuses (7). Reactivation of latent *T. gondii* infection is a concern for immunocompromised patients.

Accurate diagnosis of *T. gondii* and distinguishing between acute and chronic infection is essential for determining the appropriate treatment approach. Diagnostic methods such as IgG avidity testing can help to differentiate between the acute and chronic stages of infection (8, 9). Currently, the combination of pyrimethamine (PYR) and sulfadiazine (SDZ) is the recommended treatment for acute toxoplasmosis (10). However, these drugs have serious side effects such as neutropenia, thrombocytopenia, leucopenia, increased serum creatinine and serum liver enzymes, hypersensitivity, and bone marrow suppression (11).

Both PYR and SDZ inhibit dihydrofolate reductase and dihydropteroate synthase, essential enzymes for folate synthesis, consequently preventing the survival and duplication of *T. gondii* (12, 13). While these drugs are effective in controlling the acute phase of toxoplasmosis, they cannot completely eradicate encysted bradyzoites or treat congenital toxoplasmosis. In addition to these drugs, alternative drugs such as clarithromycin, atovaquone, azithromycin, spiramycin, dapsone, and cotrimoxazole (trimethoprim-sulfamethoxazole) are also used for the treatment of toxoplasmosis (11).

In recent years, nanotechnology-based approaches have gained prominence in biomedical research and offer promising prospects for the treatment of various diseases (14). Nanoparticles (NPs) are particularly appealing due to their unique physicochemical properties and their potential to serve as therapeutic agents. These microscopic particles typically range in size between 1-100 nm (15). Reducing the size of a material to the micro and nano range can present distinctive advantages over bulk materials (16).

Some studies have been conducted on the therapeutic potential of metal oxide nanoparticles against various pathogens, including bacteria, viruses, fungi, and protozoa (17). Zinc is an essential micronutrient that plays a critical role in numerous biological processes in the human body, including enzymatic activity like carboxypeptidase inactivation (18). Zinc oxide nanoparticles (ZnO-NPs) are widely used in industry, chemical sensors, cosmetics, and medicine due to their special biocompatibility and optical, catalytic, electrical, and antimicrobial properties (19). Because ZnO-NPs have a high surface-to-volume ratio, they represent different physical, chemical, and wide levels of biological responses (20). Despite the extensive use of ZnO-NPs in different fields, there is a paucity of information on their in vivo therapeutic efficacy (4, 21, 22). Furthermore, there is only one recent research

that has demonstrated the potential therapeutic impact of ZnO-NPs against *T. gondii* via in vivo setting (23). Building upon this emerging literature and aiming to expand our understanding of the therapeutic effects of ZnO-NPs in the context of *T. gondii* infection, our study focuses on assessing the anti-*Toxoplasma* effects of ZnO-NPs.

Saadatman et al. (24) studied the prophylactic effects of ZnO-NPs on *T. gondii* and showed promising prophylactic effects of ZnO-NPs on *Toxoplasma* infection.

According to previous studies and recognizing the lack of research on the therapeutic effects of ZnO-NPs in mice, the present study was conducted to evaluate the therapeutic effect of ZnO-NPs in BALB/c mice infected with *T. gondii* (RH strain) tachyzoites.

Materials & Methods

The present study was carried out from October 2021 to September 2022 in the Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran.

Animals

Thirty-five female BALB/c mice weighing 20-25 g and 6-8 weeks old were selected. All mice were housed in groups of seven in separate cages under standard laboratory conditions, including an average temperature of 20-25 °C, a regulated light-dark cycle, and provided ad libitum food and fresh drinking water (25).

All animal procedures were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals published by the United States National Institutes of Health (26) approved by the Ethical Committee of Tehran University of Medical Sciences, Tehran, Iran (IR.TUMS.SPH.REC.1400.212).

Parasite strain

Tachyzoites of *T. gondii,* RH strain were maintained by serial passages in the peritoneal cavity of BALB/c mice at the Department of Medical Parasitology and Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran. Approximately 0.3 ml of tachyzoites (containing 10^6 tachyzoites) were inoculated into the peritoneal cavity and harvested after 4 to 6 days. The peritoneal fluid was collected and centrifuged at 5000 rpm for 10 minutes, after three times washing, tachyzoites were counted using hemocytometry slides (27).

Synthesis of zinc oxide nanoparticles

Zinc oxide colloidal nanoparticles (ZnO-NPs), measuring 20 nm in size, were supplied at a concentration of 100 ppm from Vista Elixir Technology Development Company (Iran-108846: Lot No). The ZnO-NPs were synthesized by following previously reported methods (28), with some modifications. Specifically, 3.5 g of zinc nitrate was dissolved in 100 ml of deionized water and stirred well on a Stirrer heater at 600 rpm. Subsequently, 40 ml of the prepared plant extract was continuously stirred into the zinc nitrate for 4 hours. This resulted in the formation of a white precipitate, which was filtered, washed with deionized water three times, transferred to an oven, and maintained at 80 °C for 24 hours.

The concentrations of 10, 20, and 50 ppm were specifically chosen based on a previous master's thesis project conducted within our institution, which served as a pivotal reference point for our current study. Prior to the final selection of the dilutions, a preliminary test is conducted with various dilutions, and the suitable ones are selected accordingly (29). Additionally, our decision regarding these concentrations was further supported by findings from a study on nanosilver particles (30).

Treatment of acute toxoplasmosis

To evaluate the anti-*Toxoplasma* efficacy of ZnO-NPs, 35 female BALB/c mice were inoculated with 10^4 tachyzoites of *T. gondii,* RH strain and subsequently allocated to five

groups with seven mice in each and treated as follows: Group 1 as the negative control group, Group 2 received a 50 ppm ZnO treatment, Groups 3, 4, and 5 were treated with 10, 20, and 50 ppm ZnO-NPs, respectively.

Treatment was initiated four hours post-inoculation via oral gavage and continued daily until the mice's death. Mice were monitored daily, and the survival rate was documented for each group. In order to determine the extent of parasitic infection, peritoneal fluids from seven mice within each group were collected on the fifth day of treatment and subjected to microscopic examination for the quantification of tachyzoites. The number of tachyzoites was counted in both the treatment and control groups using light microscopy at a magnification of ×400.

Statistical analysis

Statistical analysis was carried out using IBM SPSS for Windows version 22.0 (IBM, Armonk, NY, USA). Mean and standard deviation were used to report quantitative values. Statistical differences between the treatment and control groups were analyzed using Kruskal-Wallis. The Bonferroni test was used as a post hoc test for multiple comparisons. Moreover, the Kaplan–Meier method was used to compare survival rates between the groups. *P*-values ≤0.05 were considered statistically significant.

Results

Parasite count

A statistically significant variation in tachyzoite counts was observed among the five groups on days five ($P<0.001$), six ($P<0.001$), and seven ($P=0.001$) post-infection, Table 1. The group treated with 50 ppm ZnO-NPs recorded the lowest mean count of tachyzoites compared to both the control group and other treatment groups on the 5th, 6th, and 7th days post-inoculation. The group treated with 50 ppm ZnO-NPs was the only survived group on the 9th and 10th days post-infection (Fig. 1).

Table 1: Tachyzoite counts in peritoneal fluid BALB/c mice post *Toxoplasma gondii* infection

Days post inoculation	**Tachyzoite count ± SD (×10^5)**					***P* value**
	Control	ZnO	ZnO-NPs			
			10 ppm	20 ppm	50 ppm	
Day 5	45.1±7.4	43.4±6.4	31.1±4.6	11.5±1.4	2.8±0.6	<0.001
Day 6	199.4±13.6	195±6.9	173.7±42.8	50.4±42.4	31.3±5.6	<0.001
Day 7	664±53.0	623.7±28.1	365.4±21.5	291.1±27.9	51.5±2.2	0.001

ZnO: zinc oxide; ZnO-NPs: zinc oxide nanoparticles

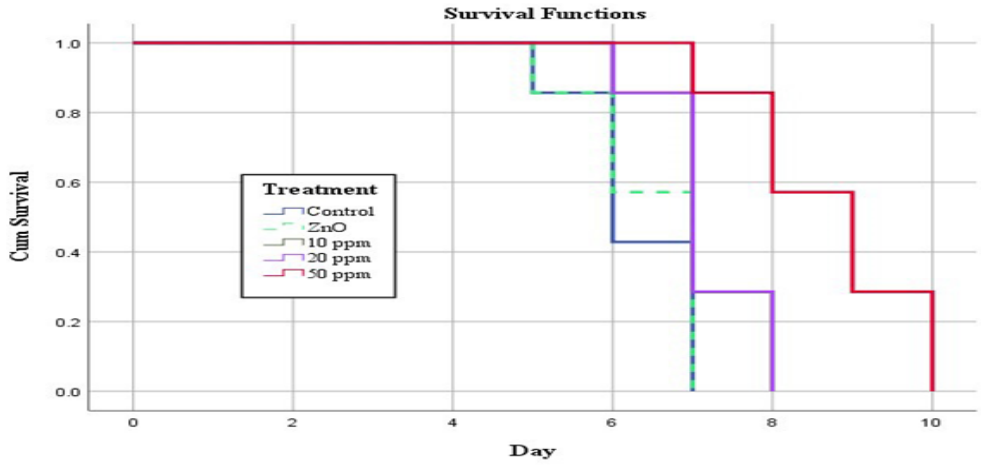

Fig. 1: Survival rates of BALB/c mice inoculated with tachyzoites of *Toxoplasma gondii* RH strain and treated with different doses of ZnO-NPs, and ZnO compared with those in the control group (P<0.0001)

Pairwise comparisons of groups, using Post hoc Bonferroni's method, revealed that on the 5th day post-inoculation, the treatment group that received 50 ppm of ZnO-NPs exhibited significantly lower counts compared to the other groups (P<0.001), in addition, the group that received 20 ppm of ZnO-NPs had significantly lower counts compared to the ZnO-treated group (P<0.001). On the 6th day post-inoculation, the group that received 50 ppm of ZnO-NPs demonstrated significantly lower counts compared to the group that received 10 ppm of ZnO-NPs (P<0.001). Furthermore, on the 7th day post-inoculation, the treatment group administered 50 ppm of ZnO-NPs recorded significant differences relative to both the ZnO-treated group and the control group (P = 0.001).

Survival rate

The mean survival time in all treated groups was significantly longer than the control group (P<0.001). The mean survival time for the control, treatment with ZnO, 10 ppm ZnO-NPs, 20 ppm ZnO-NPs, and 50 ppm ZnO-NPs was 6.2±0.28, 6.4±0.29, 7.1±0.26, 7.1±0.26, and 8.7±0.42 days, respectively.

In the control and ZnO-treated groups, infection symptoms were observed from day 4, and all mice died between 5–8 days post-inoculation. No symptoms of infection were observed in ZnO-NPs-treated mice until day 5 of infection. In the treatment groups that received 10 and 20 ppm of ZnO-NPs, infection symptoms were observed after day five, and the mice died between 6-8 days post-inoculation. Compared to the other groups, the mice that received a dosage of 50 ppm of ZnO-NPs exhibited an extended survival period of 8 to 10 days post-inoculation (Fig. 1).

Discussion

This study demonstrated that administrating ZnO-NPs reduces tachyzoite counts in infected mice compared to both the zinc oxide treated and control groups. The group treated with 50 ppm ZnO-NPs showed the lowest tachyzoite count. Furthermore, intervention with ZnO-NPs led to a significant increase in survival time. The dose of 50 ppm resulted in the longest survival time among the treated groups, surpassing those treated with 20 and 10 ppm doses.

Currently, the gold standard treatment for toxoplasmosis is the combination of pyrimethamine and sulfadiazine (10), but prolonged use of these drugs may cause hematologic and renal toxicities (22). Besides their side effects, these drugs have limitations such as insufficient absorption, poor cell membrane penetration, and drug resistance (11). Thus, the identification of safe and effective medications is urgently required. While NPs have been extensively explored as drug delivery systems and vaccines, their potential as a therapeutic modality has only been minimally investigated. The present study aimed to evaluate the therapeutic efficacy of ZnO-NPs on *T. gondii* tachyzoites in BALB/c mice.

Saadatmand et al. (24) investigated the prophylactic effects of ZnO-NPs and showed promising results, with oral administration of ZnO-NPs reducing the mean number and diameter of brain tissue cysts in mice, with complete control of the infection observed at a dose of 150 mg/kg.

To date, only one study, recently published in 2023, has addressed this topic (23). In this study, researchers used the ginger ethanolic extract to produce ZnO-NPs by combining it with sodium hydroxide and zinc acetate. These ZnO-NPs were then assessed for their effectiveness against *T. gondii* RH strain in infected mice (23). In alignment with our findings, their study similarly discovered that mice treated with ZnO-NPs exhibited a notably extended survival rate and decreased parasite burden in comparison to untreated mice or those treated with spiramycin (23).

The use of ZnO-NPs had notable impacts on the behavior of the parasites at a molecular level. The treatment caused significant deformities in the structure of the tachyzoites, potentially targeting their inner components, leading to a reduction in the parasite count in the liver (23, 31, 32).

In the present study, the therapeutic efficacy of ZnO-NPs on *T. gondii* tachyzoites was evaluated in vivo. The results showed a significant reduction in the number of tachyzoites in mice treated with ZnO-NPs, highlighting the potential of ZnO-NPs as a therapeutic agent for *Toxoplasma* infection. However, further in vivo and clinical investigations are necessary to confirm these results and explore possible mechanisms of action.

Various chemical forms, such as silver, chitosan, and selenium, have been investigated as nanoparticles to treat acute or chronic toxoplasmosis in both in vitro and in vivo experiments.

Azami et al. indicated the potential of curcumin Nanoemulsion (CR-NE) in the treatment of acute and chronic toxoplasmosis in mice. In acute toxoplasmosis, treatment with CR-NE increased the survival time of infected mice and significantly reduced the number of tachyzoites. In chronic toxoplasmosis, treatment with CR-NE resulted in a significant decrease in the number and size of tissue cysts compared to the control group, as well as a downregulation of *BAG1* gene expression. Overall, CR-NE could be a potential therapeutic option for toxoplasmosis (4).

Teimouri et al. investigated the effect of different concentrations of chitosan nanoparticles (CS-NPs) with varying molecular weights (MWs) on the mortality of *T. gondii* tachyzoites both in in vitro and in vivo experiments. The findings revealed that the anti-*Toxoplasma* activity of CS-NPs increased as their concentration increased and MW decreased. In in vitro experiments, LMW CS-NPs at 500 and 1,000 ppm concentrations and MMW CS-NPs at 1,000 and 2,000 ppm concentrations resulted in 100% mortality of tachyzoites after 120-180 minutes, whereas HMW CS-NPs only achieved this at 2,000 ppm concentration. In in vivo, the growth inhibition rates of tachyzoites in mice treated with CS-NPs were 79-86%, depending on the MW, compared to the sulfadiazine treatment group. These results suggested that CS-NPs have the potential as an anti-*Toxoplasma* agent (33).

Despite the promising results of our study and all studies mentioned above, developing effective and safe nanoparticle-based therapies for toxoplasmosis remains a challenge. Further research is necessary to understand fully the mechanisms of nanoparticle-mediated anti-*Toxoplasma* activity.

This study represents the investigation into the therapeutic potential of ZnO nanoparticles against toxoplasmosis. Nonetheless, the effectiveness of ZnO nanoparticles against other pathogenic organisms, including various protozoan parasites, has been investigated in past studies (34, 35).

In this study, our focus was primarily on the assessment of the parasite load in the peritoneal fluid to analyze directly the immediate response to the administered treatment. However, we acknowledge that the lack of calculation of the parasite load in the liver and spleen restricts our understanding of the potential systemic effects of the interventions beyond the peritoneal cavity.

Conclusion

The average survival time of the mice treated with ZnO-NPs was significantly higher compared to the control and ZnO-treated groups. Moreover, the group of mice that received a concentration of 50 ppm of ZnO-NPs exhibited the most significant results compared to the other treated groups. However, further studies are needed to evaluate the efficacy and safety of ZnO-NPs in clinical settings.

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Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

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