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Study of the efficiency of Mn_2O_3 nanoparticles in the treatment of dysentery compared to metronidazole *in vivo*

Hind A. Yousif[1*], May naji Alkhanaq[2]
[1*, 2] College of science, University of Wasit, Iraq

Abstract

Entamoeba histolytica is a protozoan parasite of medical importance that causes gastroenteritis in a variety of vertebrate hosts. The current study aimed to isolate and purify the *Entamoeba Spp.* Parasite, infect experimental mice, and confirm the therapeutics efficacy of Manganese oxide nanoparticles on the *Entamoeba histolytica* parasite *in vivo* compared to the drug metronidazole for the treatment of amebiasis. The results of the present study demonstrated the efficiency of Manganese oxide nanoparticles in treating dysentery, evidenced by a significant reduction in the parasite's cysts in the stools of infected and treated mice. The therapeutic efficiency of manganese oxide nanoparticles at concentrations of 50, 75, and 100 µg/ml, as well as a combination of 37.5 µg/ml manganese oxide nanoparticles and 25 mg/kg metronidazole, on the tenth day was 72%, 89%, 100%, and 76%, respectively, while the therapeutic efficacy of 50 mg/kg metronidazole in mice was 71%, while the positive group was lying cysts. The higher therapeutic efficiency was to Manganese oxide nanoparticles at the concentration 100 which became zero on the tenth day.

Keywords: *Entamoeba histolytica*, nanoparticles, metronidazole, parasite, therapeutic efficiency.

1. INTRODUCTION

Entamoeba histolytica, the causative agent of amebiasis, is a significant parasite contributor to illness and mortality, especially in underdeveloped nations. Approximately 50 million cases with noticeable symptoms and 100,000 fatalities occur globally each year [1]. It has a significant capacity to infiltrate and annihilate human tissue. The parasite resides in the gastrointestinal tract of humans, where the oxygen levels are lower than normal. When it invades the tissues, it comes into contact with reactive oxygen species, such as superoxide radical anions and hydrogen peroxide [2]. All infections with *E. histolytica* must be treated due to the possibility of dissemination and the development of extraintestinal symptoms.Metronidazole is the first-line therapy for intestinal amebiasis and amebic liver abscess, followed by a luminal medication [3].

It is the primary constituent found in the plasma, accompanied by smaller amounts of the 2-hydroxymethyl metabolite. Less than 20% of the metronidazole in the bloodstream is attached to plasma proteins. Both the parent drug and the metabolite exhibit bactericidal action against the majority of anaerobic bacteria strains in laboratory conditions, as well as trichomonacidal activity [4].

Nanoparticles (NPs) are particulate substances with at least one dimension less than 100 nm. The small size and large surface area of nanoparticles (NPs) enhance their colloidal stability and bioavailability, enabling them to cross blood-brain barriers, enter the pulmonary system, and attach to endothelial cells [5]. Metal oxide nanoparticles (MONPs) possess several advantageous characteristics, including straightforward preparation methods, exceptional stability, and the ability to be engineered to specific size, shape, and porosity. MONPs also exhibit consistent swelling behaviour, can be easily functionalized with various molecules, and can be incorporated into both hydrophobic and hydrophilic systems due to their negatively charged surface. These properties make MONPs highly promising for biomedical applications [6].

There are several references about the treatment of mice infecting with entamoeba histolytica. Abdel Halim et al. studied the synthesis and characterization of silver and copper nanoparticles using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and X-ray fluorescence (XRF). These nanoparticles were then used in the treatment of mice infected with entamoeba histolytica [7]. Amany EL

Sayed Saber et al. evaluated the efficacy of bee venom (BV)-loaded chitosan (CS) nanoparticles in the treatment of amoebiasis. They determined that BV has an ant amoebic effect and that orally administered BV-loaded CS nanoparticles may be a more effective alternative to subcutaneously injected BV. They also demonstrated that BV has an ant amoebic effect [8]. Lucía Margarita Valenzuela-Salas et al. investigated the comprehensive characterization of a protein-coated AgNPs formulation that was highly biocompatible, as well as its selective antitumor and amoebicidal activity [9].

This study was conducted with the intention of identifying and isolating *Entamoeba histolytica* from patients who were experiencing bloody and mucous diarrhea. The iodine stain was made use of to make the diagnosis microscopically.

2. MATERIAL AND METHODS:

2.1. Collection of stool samples

Stool samples were obtained from individuals who were receiving treatment at Al Kut Hospital and Al-Karama Teaching Hospital in the city of Kut.

Sixty-seven stool samples were marked with bloody and mucus diarrhea gathered between December 2023 and March 2024. Children with diarrhea in a different age of both sexes have samples.

The samples were collected in clean, securely sealed, and sterilized plastic bottles with a capacity of 80 ml to prevent the sample from drying and to maintain its moisture. Each stool sample was stored at 4 °C in 2.5% potassium dichromate until it was required. The samples were numbered and subsequently transported to the laboratory of the College of Science at Wasit University for laboratory testing.

2.2 Experimental Animals

A total of 42 male Balbc mice were acquired from the Drug Control Centre of the Iraqi Ministry of Health and subsequently relocated to the animal house at the College of Science, Wasit University , their ages varied from 8 to 10 weeks, and their weights ranged from 28 to 30 gm , they were housed in plastic cages with metal covers and furnished with sawdust, which was replaced twice a week , the environmental conditions were organized and controlled in terms of ventilation , temperature and illumination, which ranged from 20 to 25 degrees Celsius and it's illumination 12:12 hours of illumination: darkness persisted for the duration of the experiment , the animals were provided with an adequate quantity of water and the integrated and locally manufactured sustenance during the study period [10].

2.3 Experimental Infection

The experimental mice were administered a 1 ml dose of the infection containing 10^3 cysts/ml orally using a sterile 1ml syringe, in order to cause infection in the experimental mice [11]. After verifying that they were checked under a microscope by using methylene blue dye on a regular basis, beginning on the third day and continuing until the seventh day, in which the infection was manifested which contain the parasite's cysts.

2.4 Design of the experiment

The experimental design consisted of seven groups, each group containing six mice and all the mice(except for the negative control) injected with *E. histolytica* cysts which were isolated and prepared before at a Concentration of 1×10^3 cells/mL [11].

The first group: Mice that were treated with concentration 50 µg|ml of Mn2O3 nanoparticles.

The second group: Mice that were treated with concentration 75 µg|ml of Mn2o3 nanoparticles.

The third group: Mice that were treated with concentration 100 µg|ml of Mn2o3 nanoparticles.

The fourth group: Mice that were treated with a mixture of (37.5 µg |ml of Mn2O3 Nps +25 mg of metronidazole) day.

The fifth group: Mice that were administered metronidazole at a dosage of 50 mg/k of mice |per day.

The sixth group (positive control): Mice infected orally by the parasite and did not get any treatment

The seventh group (negatives control): Mice not affected with parasite.

Every mouse received a dose of 0.006ml of Metronidazole solution diluted with distilled water to a total volume of 0.5 ml [12]. This dose was administered daily, one hour before breakfast. The mice were administered metronidazole daily for a duration of 12 days after the infection.

2.5 Therapeutic efficacy

According to [13] ; the therapeutic efficacy of the Mn2o3 nanoparticles and metronidazole was determined by using the following equation:

$$Therapeutic\ efficacy\ (\%) = \frac{count\ of\ cysts\ \ in\ control\ group - count\ of\ cysts\ \ in\ treatment\ group}{mean\ of cysts\ count\ in\ control\ group}$$

Stool samples from mice that were infected were collected both during and at the conclusion of the treatment period to observe the quantity of cystic and active stages.

2.6 Metronidazole

Metronidazole is rapidly absorbed after being administered orally. Treatment with these medications is ineffective against luminal trophozoites, resulting in a 40% to 60% rate of parasite persistence in the colon [14]. Therefore, a regimen of the luminal drug, specifically the non-absorbable aminoglycoside paromomycin, should be provided following a treatment interval [15].

Nitazoxanide is a promising treatment for intestinal amebiasis. A dose of 500 mg twice daily for three days resulted in microscopic improvement and resolution of E. histolytica-caused diarrhea in 80% to 90% of patients (compared to 40% to 50% with a placebo) [16].

Metronidazole is associated with fewer frequent adverse effects than nausea, headache, anorexia, and metallic taste, including electrolyte imbalance, vomiting, disulfiram-like reaction to alcohol, and peripheral neuropathy [17].

2.7 Manganese oxide nanoparticles

Manganese can be found in several stable oxide forms (MnO, Mn3O4, Mn2O3, MnO2) that crystallize into a variety of shapes, Manganese oxides display a range of unique electrochemical properties depending on their defect chemistry, morphology, porosity, and textures [18].

Transition metal oxides, particularly MnO and MnO2, are excellent options for battery anodes because they have a high capacity, are inexpensive, and have less environmental impact, Manganese oxide is an essential metal oxide for technological purposes, for instance, waste-water treatment, catalysis, biomedicine, air-purification sensors, super-capacitors, and rechargeable batteries all employ manganese oxide micro- and nanostructures.

Manganese (Mn) is a brittle, black substance that occurs naturally in minerals that contain manganese due to its favorable qualities for a wide range of applications, manganese oxides (MnxOy) have attracted the most attention depending on the various manganous oxide crystal formations [18].

3. RESULT AND DISCUSSION

The therapeutic efficacy of Mn2o3 NPs, mixture of treatments in comparison to metronidazole for *E. histolytica* in white mice (*in vivo*).

Group Period	Mn2O3 NPs 50 µg|ml	Mn2O3 NPs 75 µg|ml	Mn2O3 NPs 100 µg|ml	Mixture of (37.5µg|ml Mn2O3 NPs + 25 mg|Kg metronidazole)	Infected and treated with metronidazole 50mg/kg mice/ day
The 3rd day	33 %	38%	48%	32%	36%
The 6th day	55%	67%	87%	58%	56%
The 10th day	72%	89%	100%	76%	71%
The 13th day	87%	100 Mn2O3 NPs has been shown to possess antimicrobial properties [19, 20]. %	100%	100%	83%
The 17th day	100%	100%	100%	100%	100%

The results indicate that Mn2O3 NPs, particularly at higher concentrations (100 µg/ml) were more effective in eradicating *E.histolytica* compared to metronidazole by the 10th day. Metronidazole also demonstrated a significant reduction in parasite cyst numbers compared to the untreated control group. However, its efficacy was generally lower than that of the higher concentrations of Mn2O3 NPs.

It was shown that the effectiveness of Mn2O3 NPs and metronidazole increased with time, which is indicative of a gradual decline in the parasite cysts. This indicates that Mn2O3 NPs may have stronger action against this parasite.

All treatments showed improved efficacy over time, indicating a gradual reduction in the parasite. The superior performance of Mn2O3 NPs suggests their potential as a novel and effective treatment for

E.histolytica infection. Future studies could explore combining Mn2O3 NPs with metronidazole or other drugs to potentially enhance therapeutic efficacy. Understanding the exact mechanism by which Mn2O3 NPs kill or inhibit the growth of *E.histolytica* is crucial for developing more targeted and effective treatments. As shown in the table (3-9) presents the results of the efficacy of a combined treatment using manganese oxide nanoparticles (Mn2O3 NPs) and metronidazole compared to metronidazole alone in treating E. histolytica infection in mice. The effectiveness of these treatments was assessed at various time points. The results clearly demonstrate that the combination of Mn2O3 NPs and metronidazole was more effective in eradicating E. histolytica compared to metronidazole alone. However, the combined treatment demonstrated a faster rate of parasite clearance, this could be due to different mechanisms of action with Mn2O3 NPs possibly enhancing the efficacy of metronidazole. The combination treatment offers a promising approach for the treatment of *E. histolytica* infection, potentially leading to shorter treatment durations and improved patient outcomes.

Superiority of Combination Treatment: By the 13th day, the combination treatment had achieved a 100% cure rate, indicating that it was completely effective in treating the condition in all subjects. In contrast, the metronidazole-only treatment reached a 100% cure rate only by the 17th day (see Fig. (3-4).

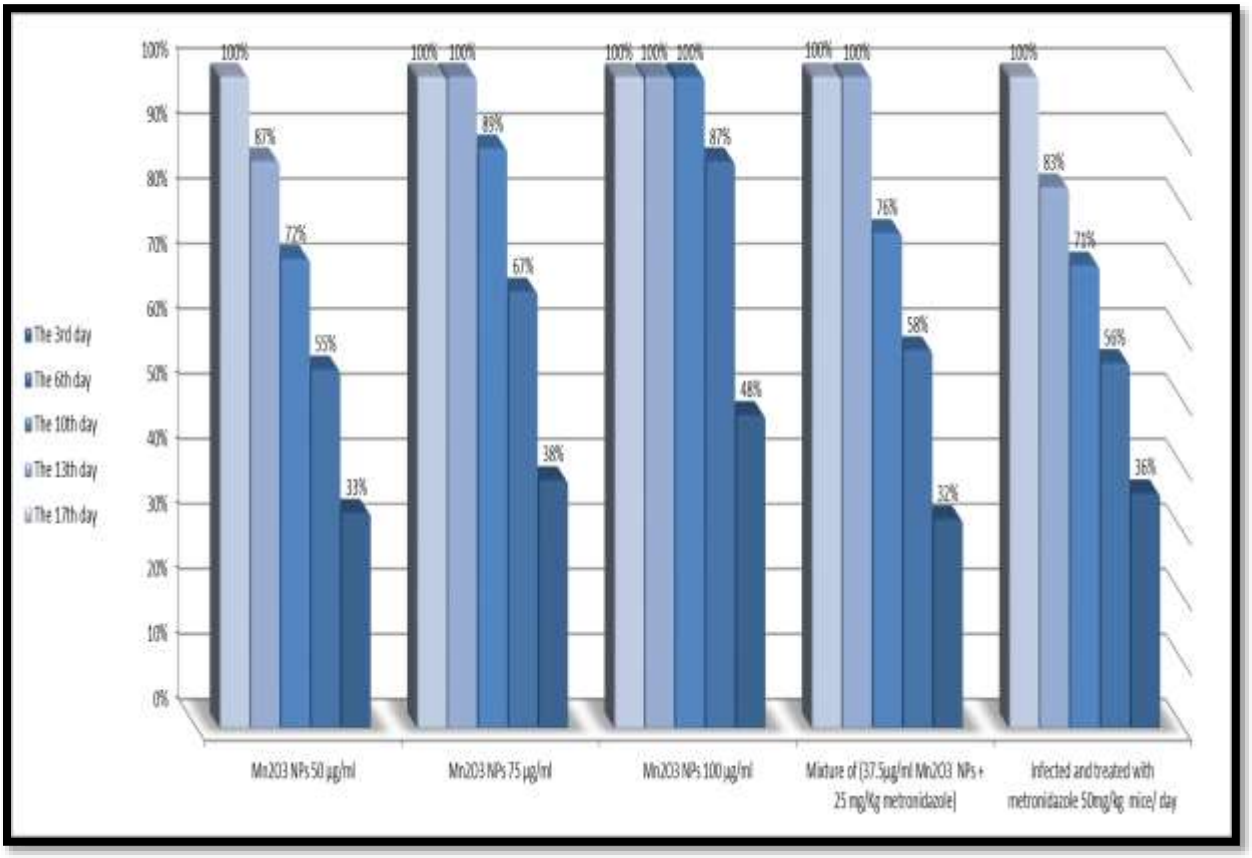

Figure (3-4): The therapeutic efficacy of Mn2o3 NPs, mixture of treatments in comparison to metronidazole for *E. histolytica* in white mice (*in vivo*).

4. CONCLUSIONS

This work underscores the remarkable therapeutic potential of manganese oxide nanoparticles (Mn_2O_3 NPs) as a viable alternative or supplementary therapy to metronidazole for Entamoeba histolytica infection. The experimental results indicated that Mn_2O_3 nanoparticles, particularly at a dosage of 100 µg/ml, markedly

surpassed metronidazole in diminishing parasite cysts, attaining total elimination by the tenth day of therapy. Furthermore, the combination of Mn_2O_3 NPs with metronidazole demonstrated synergistic effects, improving treatment effectiveness and expediting parasite clearance relative to monotherapies. The findings indicate that Mn_2O_3 nanoparticles have significant anti-amoebic efficacy and may provide an innovative, biocompatible strategy for addressing amoebiasis, especially amidst rising drug resistance and adverse effects linked to traditional therapies. Future research is advised to clarify the exact mechanisms of action, refine dosage techniques, and assess long-term safety and effectiveness in clinical models.

Ethical Approval

This research did not contain any studies involving animal or human participants, nor did it take place on any private or protected areas. No specific permissions were required for corresponding locations.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Authors contributions

All authors contributed to the study conception and design. All authors discussed the results and contributed to reading and approved the final manuscript.

Research data

No data was used for the research described in the article.

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Availability of data and materials

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