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RESEARCH ARTICLE

The Study of the Effect of Copper Nanoparticles in Enhancing the Efficacy of Metronidazole in Treating Entamoeba histolytica Infection

Taghreed A. Mustafa[1*], Prof. Dr. Intisar Ghanim Abdulwahhab[2]

[1, 2] Department of Biology, College of Education for Girls, University of Tikrit, Iraq

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***Corresponding Author:**

Taghreeda382@gmail.com

ABSTRACT

In a study conducted from May 1 to June 10, 2023, at the Samarra Pharmaceutical Laboratory, the therapeutic effects of copper nanoparticles and metronidazole were explored on mice infected with amoebic dysentery caused by Entamoeba histolytica. The mice were divided into several groups and infected with the parasite. The study focused on measuring interferon-gamma (IFN-γ) levels and conducting histological analyses of specific organs such as the liver, kidneys, and colon. The results indicated a significant increase in IFN-γ levels in the parasite-only group (Group 1, CN) (465.68 ± 36.44 pg/ml), which served as the negative control, compared to the positive control group (Group 2, CP) (78.03 ± 0.51 pg/ml). A significant decrease ($P \leq 0.05$) in IFN-γ levels was observed in the group treated with metronidazole and copper nanoparticles (Group 3) (245.34 ± 4.23 pg/ml) compared to the parasite-only group (Group 1, CN) (465.68 ± 36.44 pg/ml). Additionally, the results demonstrated noticeable improvements in immune responses and tissue conditions in the mice treated with copper nanoparticles and metronidazole compared to the positive and negative control groups.

INTRODUCTION

Diarrhea is the second leading cause of morbidity and mortality in children under the age of five worldwide. According to the World Health Organization (WHO), approximately 1.7 billion cases of diarrhea are reported annually among children, resulting in the death of 525,000 children each year. Globally, 780 million people lack access to clean water, and about 2.5 billion people live in poor sanitary conditions. Intestinal parasites are one of the main causes of diarrhea, which often becomes chronic, lasting more than three weeks. Among the most significant parasites are Entamoeba histolytica, which causes amoebic dysentery, and Giardia lamblia. Other causes of diarrhea include malnutrition, malabsorption, and contaminated water and food (Wesel et al., 2021).

Entamoeba histolytica inhabits the large intestine and appears in stool in various forms: trophozoite, precyst, and cyst (the infective stage). It causes symptomatic amoebiasis, characterized by bloody diarrhea, abdominal cramps, headache, fever, and fatigue (Matushkina et al., 2023). The trophozoite stage can invade the host's intestinal tissue, triggering a cellular immune response. The parasite's attempt to adhere to the epithelial cells lining the large intestine induces inflammation, prompting these cells to produce and release cytokines such as interleukin-2 (IL-2), which regulates immune response, interleukin-8 (IL-8), interleukin-4 (IL-4), and interferon-gamma (IFN-γ). These cytokines

act as pro-inflammatory agents, recruiting immune cells like macrophages, monocytes, and neutrophils to the infection site to attack and engulf the parasite (Yadav et al., 2020).

The World Health Organization recommends metronidazole as the preferred drug for treating Entamoeba histolytica infections. Metronidazole is a light yellowish-white crystalline powder with a slight odor, poorly soluble in water but soluble in acetone and alcohol. It is marketed under the brand name Flagyl. For adults with moderate gastrointestinal symptoms, the recommended dose is 500-750 mg three times daily. For children, the dosage is 250 mg/kg three times daily for 7-10 days. Metronidazole is the first choice for treatment due to its rapid absorption from the intestines, making it suitable for extraintestinal infections like liver, lung, and brain abscesses. Common side effects include nausea, vomiting, headache, abdominal discomfort, and a metallic taste. Rarely, it may cause severe adverse reactions such as ataxia, insomnia, and seizures (Nagaraja and Ankri, 2019).

Metronidazole (MET) is chemically known as 2-methyl-5-nitroimidazole-1-ethanol.

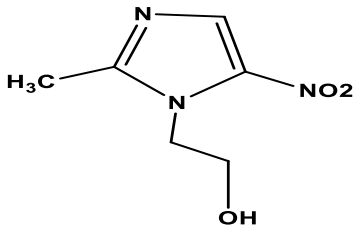


Figure 1: Structural Formula of Metronidazole (Branton et al., 2019)

C6H9N3O3

M.wt = 171.15 gm/mol

With the advancement of various sciences across all fields, the current era is considered the era of nanotechnology due to its utilization of nanoparticles in various forms in different fields such as medicine, environmental technology, and engineering (Bayda et al., 2019). Giljohann et al. (2020) and Stueber et al. (2021) indicated that nanomaterials can play a significant role in improving the chemical and physical composition of compounds and enhancing their biological efficacy when converted to nanoparticles. Each method has its advantages and disadvantages, considering that chemical methods are the most beneficial for producing homogeneous nanoparticles (Swain et al., 2021).

The objectives of the current study are as follows:

1. Testing the effectiveness of copper nanoparticles and metronidazole (Flagyl) in enhancing the treatment of experimentally induced Entamoeba histolytica infection.
2. Measuring the associated changes in certain immunological parameters after administering the infected mice with Entamoeba histolytica.

MATERIALS AND METHODS

Parasite Isolation

The parasite was isolated from stool samples obtained from patients with diarrhea, where the presence of the parasite was confirmed through direct microscopic examination. The cysts of the parasite were concentrated using the saturated sugar solution flotation method.

Parasite Diagnosis in Suspension

A drop of 0.1 ml from the top layer of the filtrate after flotation in the saturated sugar solution was placed on a clean glass slide containing a drop of Lugol's iodine solution and examined under 10X and then 40X magnification.

Identification of Infection Dose

The infection dose was determined by counting the number of cysts in 0.1 ml placed on a counting slide to establish a dose of 5000 cysts per 0.1 ml. The samples were prepared for use during administration, with the number of cysts per drop calculated to determine the infection dose, as ingestion of a single cyst can cause infection in the host.

Preparation of Aqueous Extract

After collecting and identifying the plant raw materials, they were ground into a fine powder using an electric grinder, following the method of Hernandez et al. (1994).

Determination of Metronidazole Dose

The dose was determined based on the weight of the animal, which was 25 grams, resulting in a dose of 6.5 mg/kg of a drug with a concentration of 250 mg/kg.

Animal Grouping and Experimental Design

The animals were randomly divided into three groups, each containing five animals, and placed in plastic cages, one cage per group. All cages were large, equipped with special feeders, and covered with a metal lid with a bedding of wood shavings.

Statistical Analysis

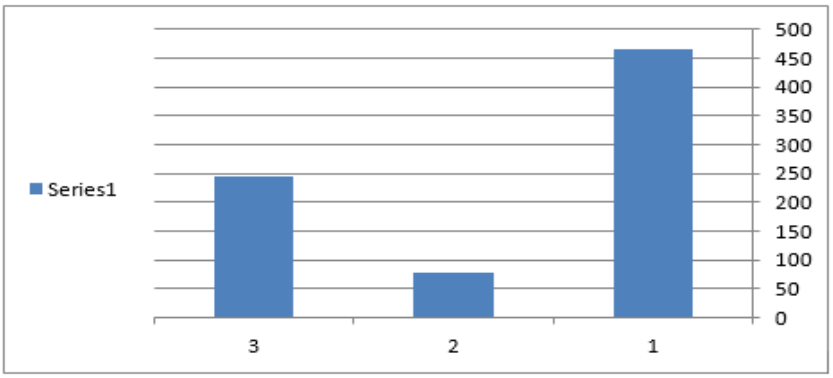
The results were statistically analyzed using the Minitab software, and the significance levels of the variables between two different sets of data were calculated using the T-test to determine significant differences at a probability level of $P \geq 0.05$.

RESULTS AND DISCUSSION

Effect of Infection on Some Immunological Parameters

4-1-6 Interferon-Gamma Level (IFN-γ Level)

The current study's results, as shown in Figure 1, indicate a significant increase in the IFN-γ level in the parasite-only group (Group 1, CN) (465.68 ± 36.44 pg/ml), which represents the negative control group, compared to the positive control group (Group 2, CP) (78.03 ± 0.51 pg/ml). The results also showed a significant decrease ($P \leq 0.05$) in the group treated with metronidazole and copper oxide nanoparticles (Group 3) (245.34 ± 4.23 pg/ml) compared to the parasite-only group (Group 1, CN) (465.68 ± 36.44 pg/ml), which represents the negative control group.



The observed effect is primarily due to the role of interferon-gamma (IFN-γ) in eliminating parasites, particularly Entamoeba histolytica. The elevated levels of IFN-γ contribute to protection against amoebiasis by stimulating macrophages, especially neutrophils, within the host's body. However,

IFN-γ cannot perform this role without immune system support and enhancement (Mortimer and Chadee, 2010).

Haque (2003) demonstrated an increase in tumor necrosis factor-alpha (TNF-α) along with IFN-γ and interleukin-2 (IL-2), produced by CD4-T cells, which collectively work against the amoeba parasite and assist in killing the active stages of the amoeba.

The current study aligns with Guo et al. (2008), who reported a significant increase in IFN-γ levels, suggesting it as a successful step toward developing a vaccine against amoebiasis. Bansal et al. (2009) found that acute amoebiasis is associated with the excessive release of pro-inflammatory cytokines in cultured environments, with increased cytokine levels in the presence of pathogenic amoeba strains and no increase with non-pathogenic strains.

This study is consistent with Rifai (2016), who investigated the immunostimulatory effect of Klebsiella pneumoniae antigens on laboratory animals infected with Entamoeba histolytica, showing a significant increase in IFN-γ levels when the immunostimulant was administered. Moreover, higher IFN-γ levels were observed with significant differences.

Moraes et al. (2015) studied the effect of IFN-γ on the functional activity of mononuclear cells (MN) during Entamoeba histolytica infection, finding variations in IFN-γ concentrations in culture media containing amoeba activators. They observed that MN cells enhanced the release of superoxide regardless of cytokine presence, while the absence of cytokines led to increased amoeba phagocytosis by immune cells. However, with IFN-γ presence, amoeba phagocytosis was reduced, indicating higher amoeba eradication rates when treated with cytokines, suggesting the therapeutic potential of cytokines.

These findings are in agreement with Ghadirian and Salimi (1993), who conducted ex vivo experiments to assess macrophage capability in killing Entamoeba histolytica using a combination of IFN-γ and 100 μg of lipopolysaccharide (LPS) derived from Mycobacterium tuberculosis on bone marrow-derived macrophages. The macrophages killed 24-60% of the amoeba within 6 hours. In vivo, TNF-β from T-cells and TNF-α from macrophages and T-cells enhance macrophage efficacy, with LPS, IFN-γ, and TNF-α indirectly exerting a cytotoxic effect on the amoeba. IFN-γ's effect on macrophages signals TNF-α. Haque et al. (2007) reported that TNF-α, IFN-γ, and IL-2 from CD4-T cells work synergistically against the amoeba, reinforcing the role of IFN-γ as a critical step in developing an amoebiasis vaccine. Their research demonstrated elevated IFN-γ, Th2 cytokines, IL-2, and TNF-α, providing protection against the amoeba. This study concurs with Guo et al. (2008), who utilized IFN-γ, IL-12, and IL-10 to develop a human vaccine against E. histolytica (Abdulwahhab, 2021).

Treated Group: Metronidazole (6.5 mg/kg) + Copper Oxide Nanoparticles (0.015 mg/kg CuO):

Liver Tissue:

Histological examination of the liver tissue from the group treated with metronidazole and copper oxide nanoparticles (CuO) revealed well-defined hepatic lobules, with a central vein in the center of each lobule that was dilated and devoid of blood. Surrounding the central vein were rows of hepatic cells and a wide network of blood capillaries connected to the central veins (Figure 1). In other histological sections, thickening of the basement membrane of the central vein and slight enlargement of clusters of hepatic cells with hypertrophied nuclei were observed. The sinusoids contained Kupffer cells (Figure 2).

Copper nanoparticles have been noted for their numerous beneficial effects in medical sciences due to their anti-inflammatory, antimicrobial, anticancer, and analgesic properties. Copper nanoparticles are highly reactive to microbes, and in recent years, they have shown a propensity to interact readily with other particles, leading to a broad spectrum of biological activities (Albalawi et al., 2021).

Our current study aligns with the findings of Lazar (2022), who used titanium dioxide in the treatment of E. histolytica infections.

Kidney Tissue:

Histological examination of the kidney tissue from the group treated with metronidazole and copper oxide nanoparticles (CuO) revealed normal-shaped glomeruli in the kidney cortex, surrounded by the Bowman's capsule and proximal and distal convoluted tubules. Some tubules contained exfoliated epithelial cells, and there was detachment and disintegration of the fibers in the capsule surrounding the kidney (Figure 3). Additionally, there was infiltration of white blood cells on the glomerular surface, with exfoliation of some lining cells in the distal convoluted tubules (Figure 4).

These findings are consistent with those of Lazar (2022), who also used titanium dioxide in the treatment of E. histolytica infections and observed tissue repair in the kidneys, as noted by Shakir and Abdulwahhab (2019).

Intestinal Tissue:

Histological examination of the small intestine tissue from the group treated with metronidazole and copper oxide nanoparticles (CuO) showed no adverse effects on the intestines. The intestinal villi were lined with simple columnar cells, with microvilli on the cell surfaces. Goblet cells secreting mucus were interspersed among the columnar cells, and the core of the villi was continuous with the intestinal glands in the basal lamina (Figure 5). The basal lamina was filled with closely packed intestinal glands lined with pyramidal cells secreting enzymes, and white blood cells extended into the submucosal layer adjacent to the smooth muscle layer. The muscular layer was surrounded by the tunic serosa, lined with simple squamous cells, free of any tissue lesions (Figure 6).

The presence of infection and the penetration of the trophozoite stages into the intestinal tissues, causing various histological changes, can be attributed to the parasite's tissue invasion mechanism. This involves three key stages: colonization, adherence to the host's epithelial cells, and the effect of intestinal motility, the presence or absence of normal flora, and the amoeba's ability to adhere to mucosal cells. Entamoeba histolytica is the only parasite capable of lysing host tissues, hence its name, histolytic amoeba.

These findings are in agreement with those of Rifai (2014), who observed similar pathological changes. Additionally, lymphocyte infiltration was noted in the colon tissue sections, which aligns with Khazali (2014), who also observed an increase in lymphocytes in cases of E. histolytica infection.

Copper oxide nanoparticles combined with metronidazole did not show any negative effects on the intestinal lining tissues, and in fact, may have enhanced their function and tissue organization.

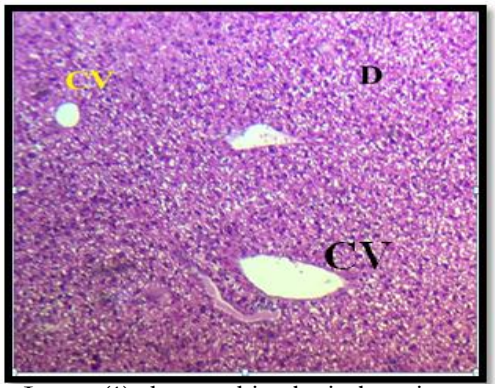


Image (1) shows a histological section of a liver treated with metronidazole + copper oxide nanoparticles (CuO), illustrating the central vein (CV) and hepatocytes (D) (H&E X 10).

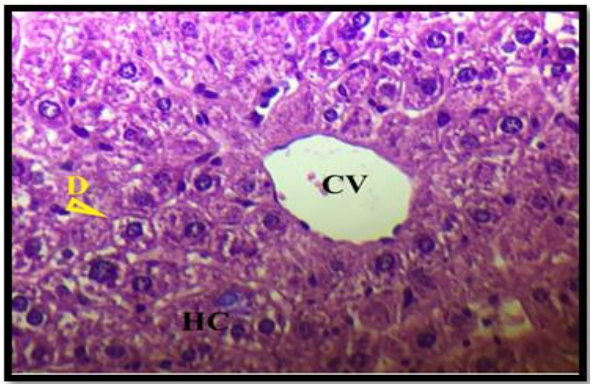


Image (2) shows a histological section of a liver treated with metronidazole + copper oxide nanoparticles (CuO), illustrating the central vein (CV), hepatocytes (HC), degeneration, and

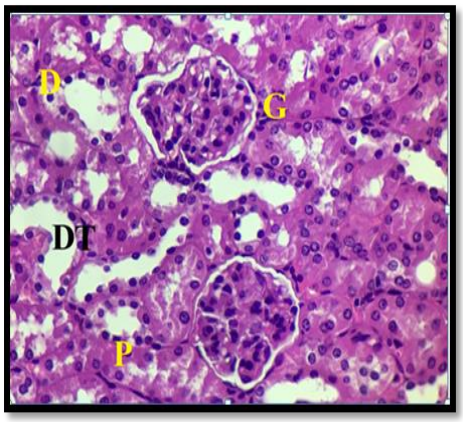


Image (3) shows a histological section of a negative control liver (parasite), illustrating the central vein (CV), general necrosis in hepatocytes (N), fatty vacuolation

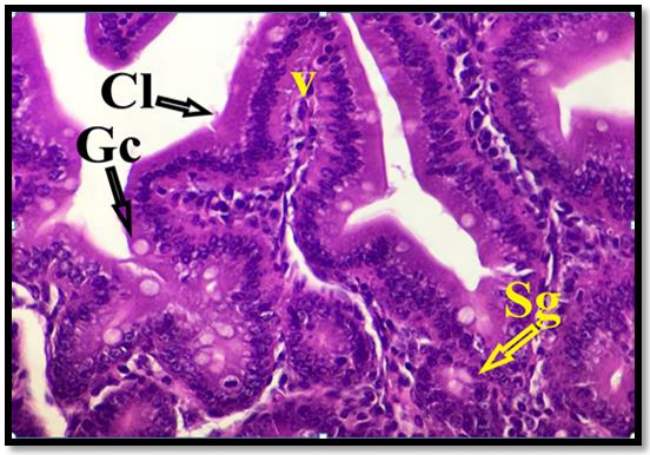


Image (4) shows a transverse histological section of the small intestine treated with metronidazole + copper oxide nanoparticles (CuO), illustrating the intestinal glands (Sg), the muscular layer (M), and the serous layer (A) (H&E X 40).

CONCLUSION

From the current study, the following conclusions were drawn:

1. Nanotechnology has paved the way for potential clinical trials to use it as a drug for treating parasites. The study demonstrated the therapeutic effect of copper oxide nanoparticles on the tissues (intestines, liver, kidneys) of experimental animals infected with amoeba at certain concentrations.
2. The concentration of 0.015 mg/kg was found to be the optimal dose of copper oxide nanoparticles, showing better therapeutic effects compared to higher concentrations. Nanoparticles exhibit a clear treatment trend that depends on dose concentration.

Recommendations

In light of the current study, the following recommendations are proposed:

1. Conduct experimental immunological studies on laboratory mice infected with Entamoeba histolytica and measure concentrations of other immune cells.
2. Measure the impact of amoebic dysentery infection on certain hormones and proteins in infected individuals compared to a control group.
3. Investigate the effects of amoebic dysentery infection on other biochemical and immunological aspects.
4. Use other nanomaterials to determine their therapeutic effects.

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