AMJ
ISSN: 0005-2523

ISSN: 0005-2523
Volume 62, Issue 05, July, 2022

Detection of histological changes *Invivo* in mice infected with *Entamoeba histolytica* and treated with Titanium Dioxide Nanoparticles

Lima Tariq Yoash Lazar[1*], Shihab Ahmed Al-Juboury[2], Mohammed Nadhir Maaroof[3]

Department of Basic Nursing Sciences, College of Nursing, University of Kirkuk, Kirkuk, Iraq[1]
Department of Biology, College of Education for pure sciences/Tikrit university, Tikrit, Iraq[2]
College of Medicine /Tikrit university[3]

Corresponding author: 1*

Keywords:
Entamoeba histolytica; Titanium dioxide Nanoparticles; Histological changes.

ABSTRACT

The present study included microscopic and molecular diagnosis of *Entamoeba histolytica* in the stool samples of patients attended to Al Alawia Teaching Hospital and Central Child Teaching Hospital in Baghdad Governorate, Iraq for the period from 1st October 2020 to 1st August 2021. The fecal samples were examined microscopically by a direct wet mount then the fecal samples were molecularly examined using specific primers to detect four virulence factors possessed by the parasite *E. histolytica*. Titanium dioxide nanoparticles were synthesized from Pseudomonas aeruginosa which producing *Pyocyanin pigment* as a reducing agent to form Titanium dioxide nanoparticles. The biosynthetic titanium dioxide was characterized using several tests (XRD, scanning electron microscopy FE-SEM, Fourier transform infrared spectroscopy, FTIR, UV visible spectroscopy). Histological changes in mice infected with *E. histolytica*, microscopic examination revealed severe amoebic colitis, which was characterized by thickening of the mucous layer with epithelial sloughing and the appearance of small and multiple ulcers in mucous layer as well as bleeding and congestion. Trophozoites were also observed in the enlarged sections in the remnants of sloughed and necrotic mucosal and epithelial cells. Our results showed that treatment with biosynthesized Titanium dioxide nanoparticles has a significant role in restoring the normal appearance of colonic epithelial and mucous cell so this shows the effectiveness of this Nanomaterial as an antiparasitic in curing the genes responsible for parasite virulence factors and thus reducing pathogenicity.

1. Introduction

Entamoeba histolytica is one of the protozoan intestinal parasite that cause amoebiasis and it ranks third among the parasites that cause deaths in the world after schistosomiasis and malaria. It affects about 50 million people annually in the world, and leads to the lives of nearly 100,000 annually. The infection

spreads all over the world, and poses a threat to health in tropical and sub-tropical developing regions as well as in developed countries [1]. Epidemiological studies indicated that 10% of people infected with *E. histolytica* show symptoms of disease while approximately 90% do not show symptoms but they are carriers [2]. *Entamoeba histolytica* possesses many virulence factors that help to penetrate and invade tissues, which contributes to an increase in pathogenesis, including the ability of the parasite to secrete enzymes, the most important of which are Active cysteine proteinase and Phospholipase, as well as the binding factor for lectin Galactose/N-acetyl-D- Galactosamine lectin (Gal/ Gal NAC) and Amoebapore [3]. Furthermore, there are other secondary factors that contribute to the development of the disease, including the amoeba strain that causes the disease and the natural and acquired immunity of the host against this strain. The nature of the host as well as interference with the normal flora in the intestine and the genetic susceptibility of the host and other factors such as: malnutrition, gender and age [4]. Nanotechnology is a modern technology that transforms large molecules into nano-sized particles 1 - 100 nanometers [5]. There are many nanoparticles that differ in their sizes, shapes, surface area and function. Metal nanoparticles and metal oxides are of great importance due to their specialized qualities in fighting microbial communities [6].

These days, Titanium dioxide Nanoparticles (TiO_2NPs) has become the focus of researchers, in addition to being approved by Food and Drug Administration (FDA). It has great biological effect in environmental purification, pharmaceutical applications, solar energy cells in addition to its ability to photocatalysis [7]. There are many methods for the synthesis of titanium dioxide nanoparticles such as physical methods, chemical methods and biological methods. Recently, biological methods are considered the most appropriate methods because they are environmentally friendly and have a low cost compared to other methods. Biosynthesis depends on redox reactions, and microbial enzymes, plant pigments and microbial components are reducing agents to give the required nanomaterial [8]. During recent years, the antimocrobial activity of nanoparticles has been proven, as AgNPs have been used against *Escherichia coli* and *Staphylococcus aureus*. Its antiparastic efficacy was also proven if [9], used commercially manufactured silver nanoparticles to know the extent of its toxicity and it's *in vitro* effect on the Trophozoite of *E. hisolytica*. A significant difference was observed in the decrease in the number of Trophozoites after incubation with silver nanoparticles. At the end the main aims of the present study is to know the effect of biosynthetic Titanium dioxide nanoparticles on histological changes in colon of mice infected with *E. histolytica.*

2. Materials and Methods

2.1 Collection and diagnosis of E. histolytica specimens

Twenty five fecal samples were collected for children infected with *Entamoeba histolytica* from the patients attending Al Alawia Children's Teaching Hospital and Central Child Hospital in Baghdad who were suffering from moderate to severe diarrhea, and the samples were examined microscopically by direct wet mount using Lugol's iodine and concentration method (floatation method).

2.2 Laboratory animal infections

The experiment was conducted on (30) adult Wister albino mice, aged (6-8) weeks, weight (25±2) gm, and male sex only, obtained from the Center for Drug Control/Baghdad. The mice were housed in the animal house of the Biotechnology Research Center / Al-Nahrain University. The mice were placed in plastic cages, and they were continuously provided with sterilized drinking water in the relevant bottles and sterile feed obtained from the same institute with adequate temperature and ventilation.

ISSN: 0005-2523
Volume 62, Issue 05, July, 2022

Feces of mice were examined before starting the experiment to ensure that they were not infected with any intestinal parasites and then five mice were killed as they were considered as a negative control group. As for the rest of the group (25) mice, they were infected orally with amoebic suspension using Gavage and the examination and confirming the infection molecularly, five mice were taken and their stool samples were collected and stored in DNA/RNA Shield (Fecal Collection tube) and considered as a positive control group and were not treated with any treatment.

For instance for the remaining (20) mice, they were divided into four groups that were treated with intraperitoneal injection (IP) with four different concentrations (50, 75, 100, 150) µg/ml of biosynthesized Titanium Dioxide Nanoparticles.

3. Results

3.1 Pathophysiological changes in experimental animals infected with E. histolytica

Results of the macroscopic examination of mice infected with *E. histolytica* showed congestion and enlargement of the internal organs, especially the cecum and colon. As well as, intestinal congestion and swelling with green discoloration was observed as shown in Figure 1. Which indicates the death of cells and congestion of blood vessels in the serous layer and because of the slow movement in these areas, which gives the parasite an opportunity to attack the mucous layer of the intestines by its decomposing enzymes and causes its perforation and transmission to other organs such as the liver, kidneys and other organs, causing abscesses in those organs This is agree with the results of [10], [11] which indicated the occurrence of macroscopic changes and congestion in the internal organs of mice infected with *E. histolytica*.

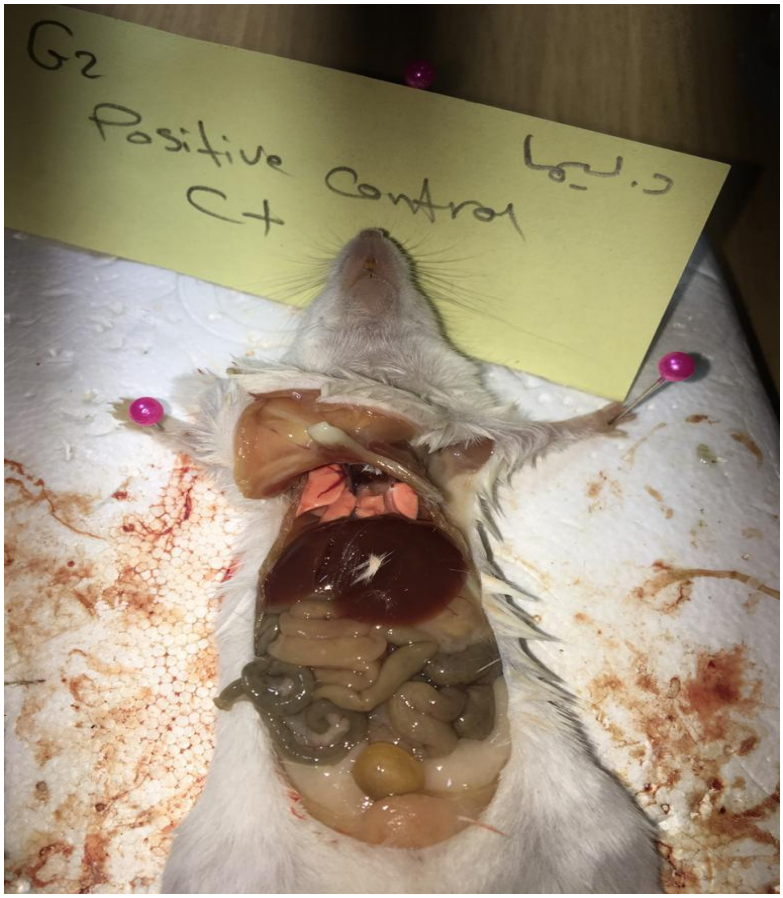

Figure 1. Enlargement and congestion of internal organs in a mouse infected with *E. histolytica*

3.2 Negative control group and positive control group (Group 1 and 2)

Since the colon is the habitat of *E. hisolytica*, when comparing the histological sections in the negative control group Figure (2) and the positive control group (infected with parasite without treatment) the results showed in the positive control group Severe amoebic colitis, which was characterized by thickening of the mucous layer with epithelial sloughing and presence of small and multiple ulcers in the mucous layer as well as bleeding and congestion, as observed in the enlarged sections, and the presence of Trophozoites in the remnants of sloughed and necrotic epithelial and mucous cells as in Figure (3) and (4) respectively.

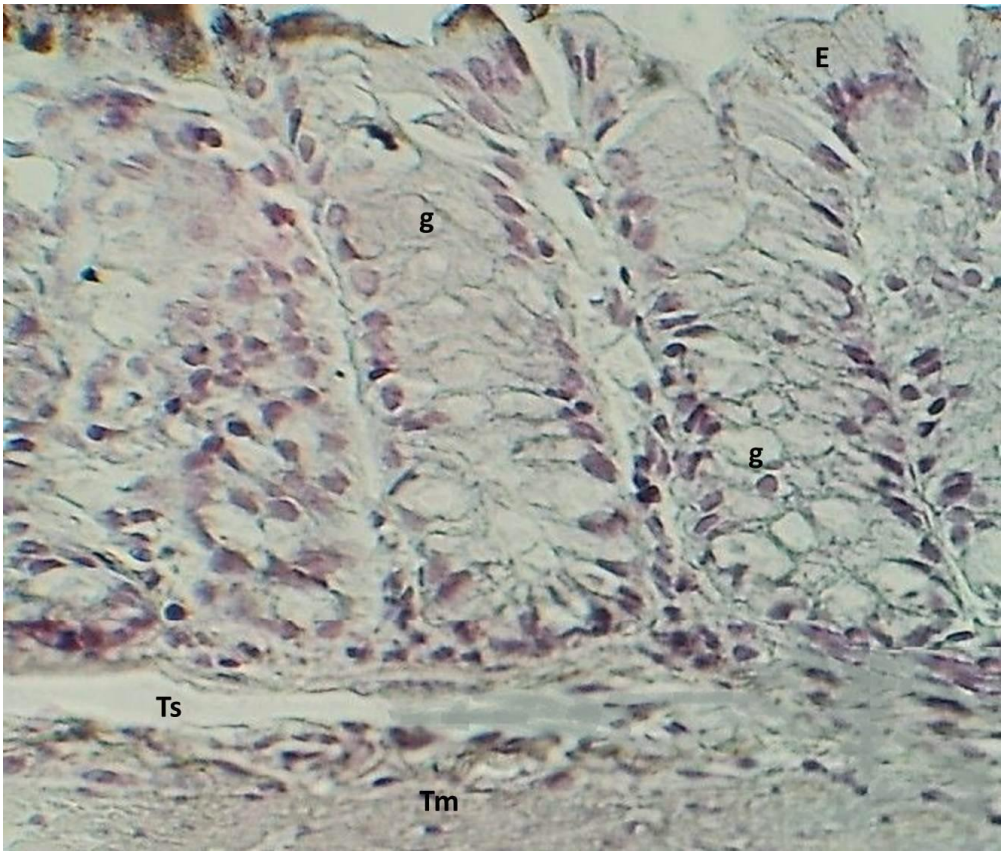

Figure 2: Section of the colon (G1 negative control group) showing normal mucosal and epithelial cells (E), goblet cells (g), submucosal layer (Ts), and muscular layer (Tm). H&E stain 400 x.

ISSN: 0005-2523
Volume 62, Issue 05, July, 2022

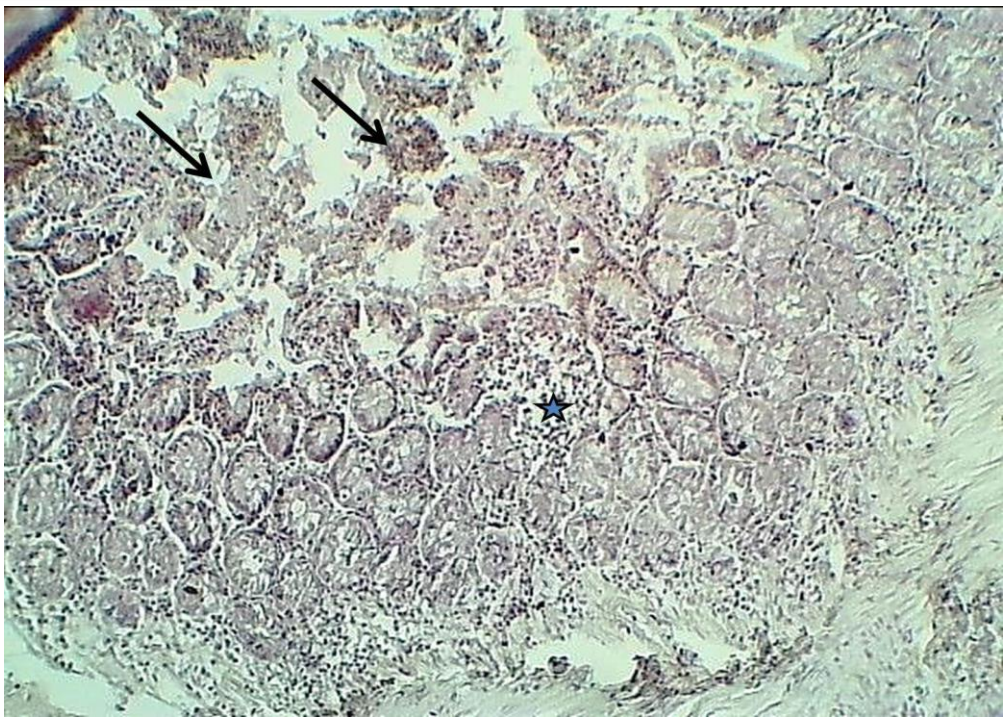

Figure 3: Section of colon (positive control) shows: Amebic colitis (arrows), which characterized by mucosal thickening, epithelial sloughing and ulcerative colitis (asterisk). H&E stain.100x

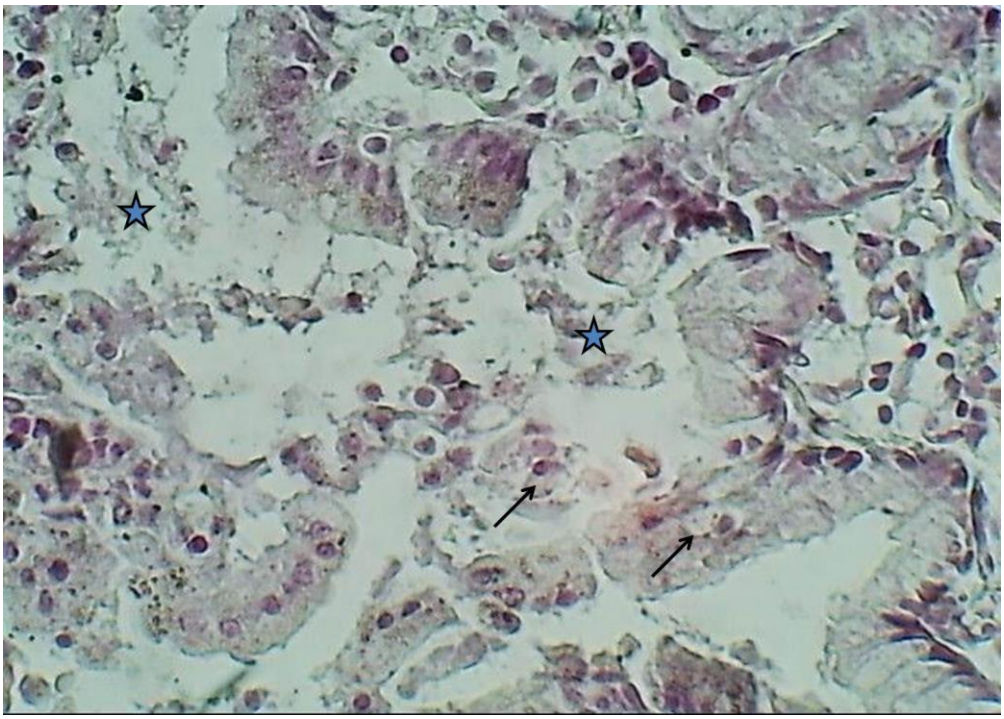

Figure 4: Section of colon (Control positive-G2) shows: Trophozoites of E. histolytic (arrows) within necrotic debris (Asterisks) & mucosal epithelial sloughing. H&E stain.400x

3.3 Third and forth group treated with 50 and 75 µg/ml Titanium dioxide nanoparticles

When comparing colon sections of the third and fourth groups treated with 50 and 75 µg/ml (TiO_2NPs) with the positive control group, it showed the proliferation of lymphocytes with the normal cellular composition of the epithelial cells and tubular glands of the colon as shown in Figure (5) and (6) respectively.

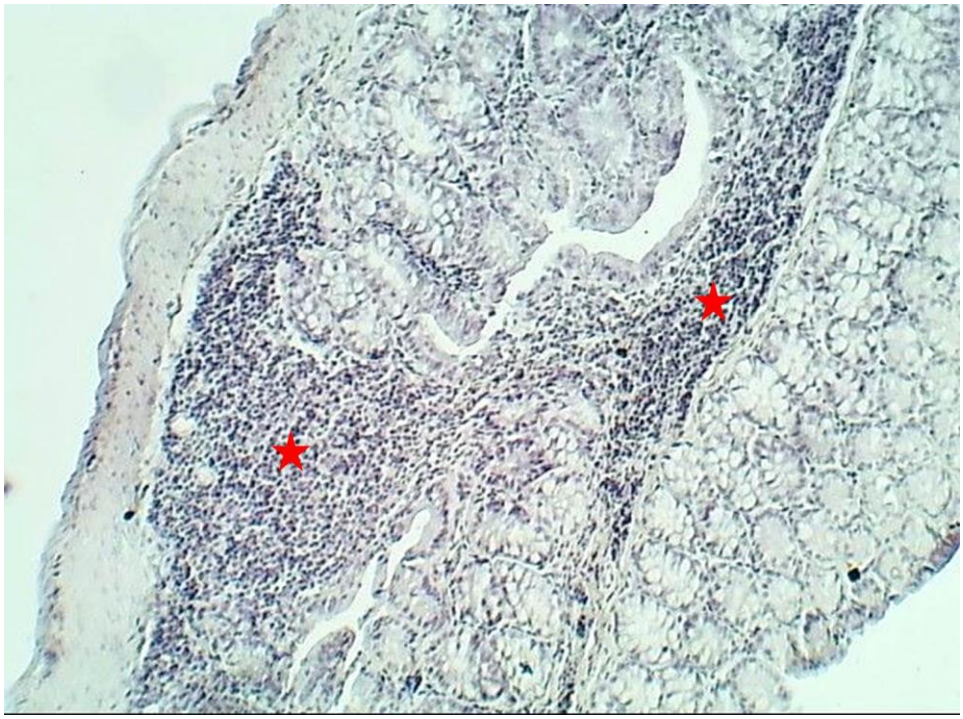

Figure 5: section of colon (third group treated with 50 µg/ml) shows: diffused infiltrative of lymphocytes (asterisks) with normal appearance of the epithelial and mucous cells and tubular glands of colon. H&E stain.100x.

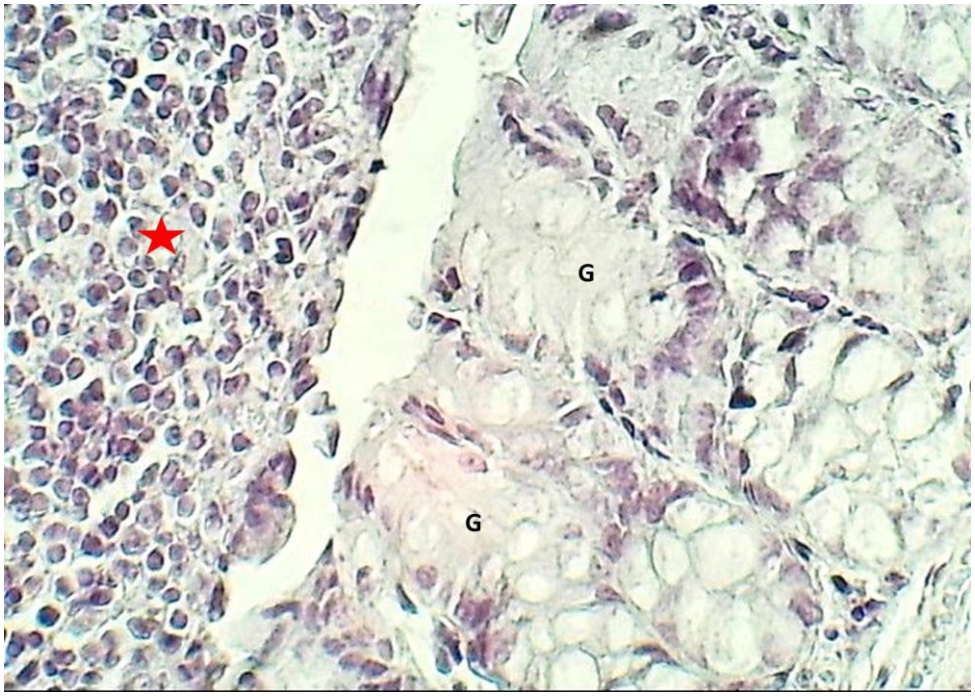

Figure 6: section of colon (forth group treated with 75 µg/ml) shows: lymphocyte proliferation (asterisks) With a normal appearance of the epithelial cells, mucosa and tubular glands (G) of colon. H&E stain.400x.

3.4 Fifth group treated with 100 µg/ml of Titanium dioxide nanoparticles

When comparing the colon sections of the fifth group treated with a concentration of 100 µg/ml of Titanium dioxide nanoparticles and the positive control group, the infected sections and treated with this concentration of nanoparticles appear similar to the negative control group. The normal thickness of the

ISSN: 0005-2523
Volume 62, Issue 05, July, 2022

mucous layer and tubular glands appears in the colon, as shown in Figure 7.

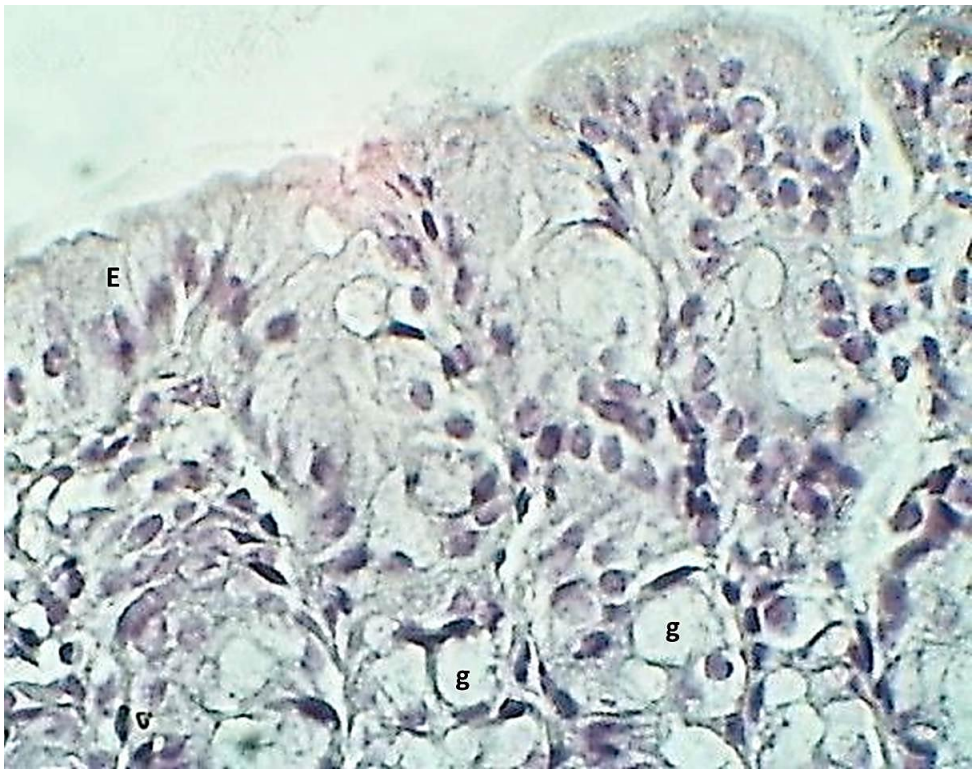

Figure 7. A section of the colon (fifth group treated with 100 µg/ml) showing lymphocyte proliferation with normal appearance of epithelial and mucosal cells (E) and tubular glands (G) in the colon. H&E stain. 400x

3.5 Sixth group treated with 150 µg/ml Titanium dioxide nanoparticles

When comparing the sixth group with the positive control group, Histological sections of the affected colon in infected mice treated with 150 µg/ml of Titanium dioxide nanoparticles showed slight thickening of the mucosal layer, swelling and degeneration of the colonic glands as shown in Figures 8 and 9 respectively.

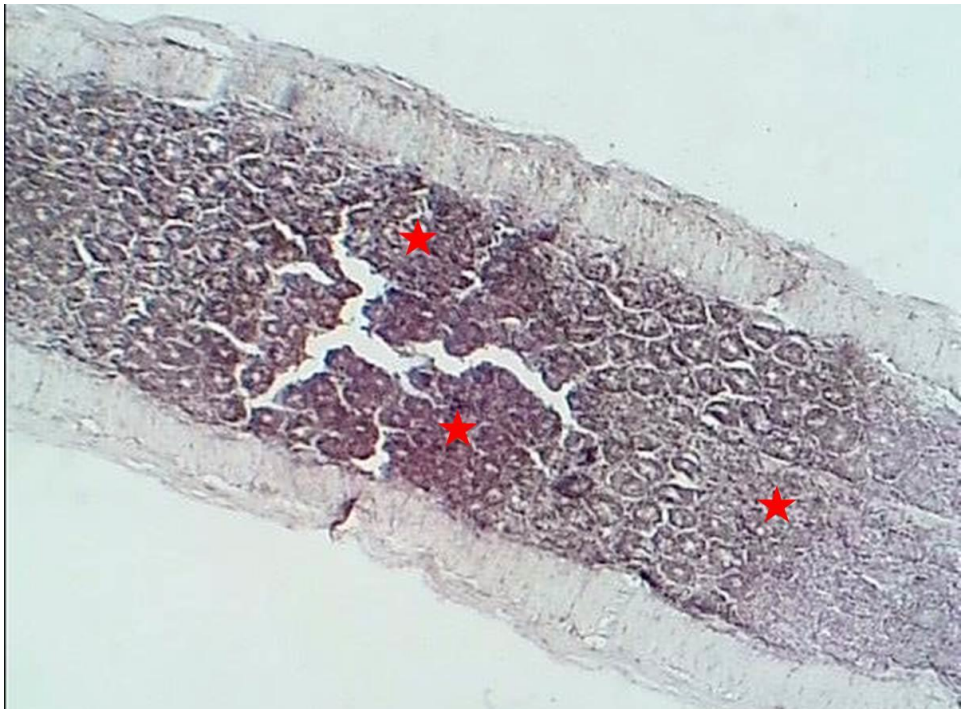

Figure 8. A section of the colon (sixth group treated with 150 µg/ml) showing the thickness of the mucous layer (asterisks). H&E stain. 100x

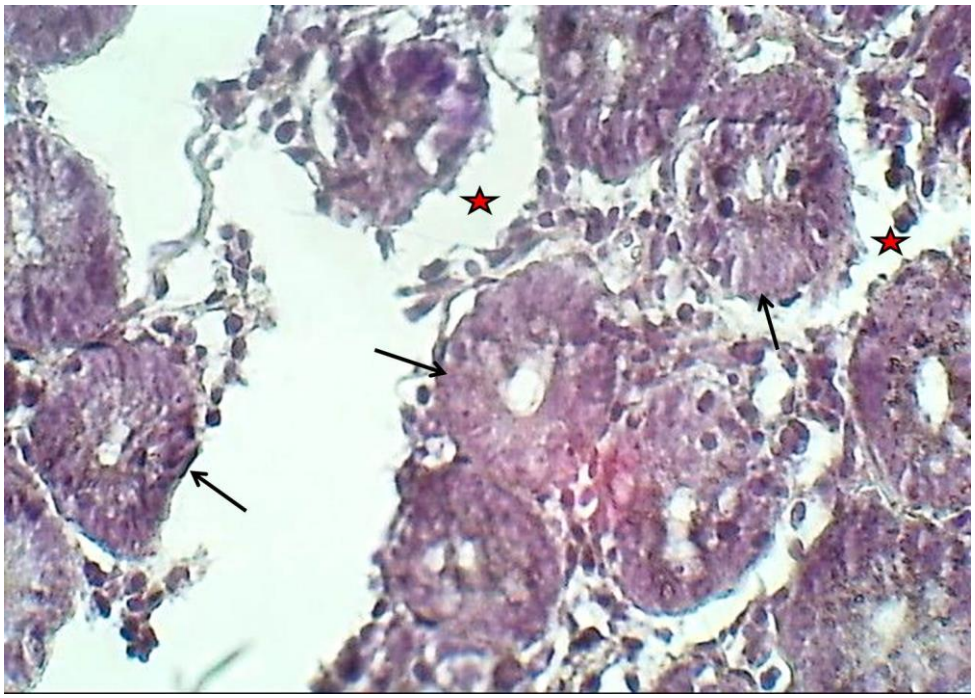

Figure 9. A section of the colon (the sixth group) shows: mucosal edema (asterisks) and degenerative changes of colon glands (Arrows) H&E stain.400x

4. Discussion

The results of the histological examination in colon of mice infected with the parasite *E. histolytica* and untreated showed the formation of small and multiple ulcers in the mucous layer and the occurrence of bleeding and congestion in the blood vessels, necrosis and erosion of the epithelial cells, the appearance of a number of sloughed cells in the intestinal cavity and the infiltration of inflammatory cells, these results of

ISSN: 0005-2523
Volume 62, Issue 05, July, 2022

this study are in agreement with [11], [12] in the occurrence of edema and ulceration in the mucous layer of the colon as well as the cecum.

As the infection and the penetration of the nutritional phases into the intestinal tissues cause different histological changes It is attributed to the mechanism of tissue invasion by the parasite Which includes three important stages: first, colonization second, association with the host's colonic epithelial cells and lysi and third, "destruction and loss of the mucosal layer." The ability of the amoeba to adhere to colonic mucosal cells. The tissue amoeba is the only parasite among the genus amoeba that has the ability to lysis tissues of the host, hence the name tissue amoeba. The results of this study also agreed with [13] in the reveal of above pathological changes. These pathological tissue changes are due to the fact that after reaching the intestine, the histolytic amoeba parasite begins to divide and double its number, then sticks to the mucous membrane and degrades the tissue by the action of enzymes possessed by the feeding phase Cysteine proteinase Thus, it leads to an inflammatory state, as during the penetration process, the parasite kills and devours the epithelial cells and red blood cells, as the parasite has (Myocin IB) in the pseudopodia, In the vesicles and in the plasma membrane of the active phase, as this substance plays an important role in the process of vegetation, as it surrounds the material to be ingested and adapts the form of vegetation around the phagocytic body, and this is consistent with what was indicated by [14].

The spread of lymphocytes was also observed in the histological sections of the colon, and these results agreed with [12] who also noticed an increase in lymphocytes in the case of infection with *E. histolytica*. As for treatment with biosynthesized TiO2 NPs, it has a significant role in restoring the normal appearance of the colonic epithelial and mucous cells naturally when using concentrations of 50, 75, and 100 µg/ml. This shows the effectiveness of titanium dioxide nanoparticles as an antiparasitic in neutralizing the genes responsible for parasite virulence factors and thus reducing pathogenicity. When using a concentration of 150 µg/ml, it was observed in the tissue sections of the colon that the mucosal layer thickened, and the dissolution and necrosis of the colonic glands.

5. References

[1] Al-Areeqi MA, Sady H, Al-Mekhlafi HM, Anuar TS, Al-Adhroey AH, Atroosh WM.,et al. First molecular epidemiology of Entamoeba histolytica, E. dispar and E. moshkovskii infections in Yemen: different species-specific associated risk factors. (2017) . Trop Med Int Health 22(4):493–504

[2] Roure S, Valerio L, Soldevila L, Salvador F, Fernández-Rivas G, Sulleiro E., et al.Approach to amoebic colitis: epidemiological, clinical and diagnostic considerations in a non-endemic context (Barcelona, 2007-2017). (2019) . PloS One. 14(2):0212791

[3] Rostami NM, Dabiri H, Zali MR, Kazemi B, Haghighi A. Discrimination of Entamoeba moshkovskii in patients with gastrointestinal disorders by single-round PCR. Jpn. J. Infect. Dis.(2010); 63(2) : 136-138.

[4] Loh, R. K. Vale, S. and McLean-Tooke, A. (2013). Quantitative serum immunoglobulin tests. Australian family physician, 42(4), 195.

[5] Dos Santos RMA ,Da Silva P B, Spósito L, De Toledo LG, Bonifácio BV, Rodero CF,Dos Santos KC, Chorilli M, Bauab T M. Nanotechnology-based drug delivery systems for control of microbial biofilms: a review . Int J Nanomedicine. (2018) ; 13:1179-1213.

[6] Raghunath A, Perumal E. Metal oxide nanoparticles as antimicrobial agents: a promise for the future . Int. J. Antimicrob. Agents. (2017); 49(2):137-152.

[7] Mishra V, Sharma R, Dut Jasuja.N , Gupta DK.. A review on green synthesis of nanoparticles and evaluation of antimicrobial activity . International Journal of Green and Herbal Chemistry.(2014);3(1);081.

[8] Nithya R, Ragunathan R.. Synthesis of silver nanoparticle using Pleurotus sajor caju and its antimicrobial study. Digest journal of nanomaterials and Biostructures. (2009); 4(4): 623-629.

[9] Ahmed ZA , Mustafa TH A, Ardalan NM, Idan E M . In Vitro Toxicity Evaluation of Silver Nanoparticles on Entamoeba histolytica trophozoite. Baghdad Sci.J. (2017); Vol.14(3).

[10] Zhen Y, Zhang H .NLRP3 inflammasome and inflammatory bowel disease. Front Immunol. (2019). 10(276):1–10.

[11] Cornick S, Moreau F, Chadee K Entamoeba histolytica cysteine proteinase 5 evokes mucin exocytosis from colonic goblet cells via αvβ3 integrin. PLoS Pathogens. (2016) . 12(4):1005579.

[12] Ghosh S, Padalia J, Moonah S Tissue destruction caused by Entamoeba histolytica Parasite: Cell Death, Inflammation, Invasion, and the Gut Microbiome. Curr Clin Microbiol Rep. (2019) . 6(1):51–57

[13] Hardin RE, Ferzli GS, Zenilman ME, Gadangi PK, Bowne WB Invasive amebiasis and ameboma formation presenting as a rectal mass: an uncommon case of malignant masquerade at a western medical center. World J Gastroenterol. (2007) . 13(42):56595661

[14] Tavares,P. Rigothier, M. Khun, H. Roux, P. Huerre،M. Guillen, N.Roles of cell adhesion and cytoskeleton activity in Entamoeba histolytica pathogenesis: a delicate balance. Infect.Immun. . (2005).73:1771-1778.