2024, 21(3): 0850-0866
https://dx.doi.org/10.21123/bsj.2023.7205
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Molecular detection of the ability of Biosynthesized Titanium dioxide nanoparticles to curing some genes of virulence factors of Entamoeba histolytica

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

[1]Department of Basic Nursing Sciences, College of Nursing, University of Kirkuk, Kirkuk, Iraq.
[2]Department of Biology, College of Education for pure sciences, University of Tikrit, Tikrit, Iraq.
[3]College of Medicine, University of Tikrit, Tikrit, Iraq.
*Corresponding Author.

Received 17/03/2022, Revised 24/02/2023, Accepted 26/02/2023, Published Online First 20/08/2023, Published 01/03/2024

Abstract

The present study included the microscopic and molecular identification of *Entamoeba histolytica* by using specific primers to detect four virulence factors possessed by *Entamoeba histolytica*. Virulence factors included Active Cysteine proteinase, Galactose/N-acetyl-D-galactose-lectin, Amoeba pore C and Phospholipase. Titanium dioxide nanoparticles (TiO_2NPs) were synthesized from *Pseudomonas aeruginosa* which producing Pyocyanin pigment as a reducing agent to form it. After that we studied the ability ofTiO_2NPs to inhibit virulence factors production and curing the genes responsible for encoding them by using four different dose 2 ,3, 4, 6 mg/Kg and administered by intraperitoneal injection to laboratory mice *Mus musculus*. The infection was molecularly confirmed, then the treatment for a period of ten days, as it was given on the third, fifth, seventh, and tenth days. The results of the study showed the inability of dose 2, 3 mg/Kg of TiO_2NPs to curing the gene responsible for active cysteine proteinase and Gal/Gal NAC lectin upon treatment on the third to the tenth day, but was able to curing the genes when using dose (4,6) mg/Kg, Statistical analysis was performed , it was found that there were significant differences between the use of different dose and days of treatment. While Amoeba pore C, it was found that the four dose did not affect the inhibition on the third day of treatment. While dose 4,6 mg/Kg were able to curing the gene responsible for its encoding. Statistical analysis showed significant differences between the use of TiO_2NPs and the days of treatment. Finally, the results showed that the four doses do not affect on phospholipase. Once, performing the statistical analysis it was found that there were no significant differences.

Keywords: Curing genes, *Entamoeba histolytica*, Pyocyanin pigment, *Pseudomonas aeruginosa*, TiO_2NPs, Virulence factors.

Introduction

Entamoeba histolytica is one of the protozoan intestinal parasites that cause amoebiasis, whether symptoms appear or not, and it ranks third among the parasites that cause death in the world after

2024, 21(3): 0850-0866
https://dx.doi.org/10.21123/bsj.2023.7205
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

schistosomiasis and malaria, as nearly 50 million people are infected and it kills about 100,000 people annually in the world[1].

Epidemiological studies indicated that 10% of people infected with *E. histolytica* show symptoms of disease while approximately 90% of patients are asymptomatic [2].

Entamoeba histolytica possesses many virulence factors that help to penetrate and invade tissues, which contributes to an increase in pathogenesis, including the ability to secrete enzymes, includes active cysteine proteinase and Phospholipase, as well as the binding factor for Galactose/N-acetyl-D-galactosamine (Gal/ Gal NAC) lectin, amoebapore and phospholipase [3].

Nanotechnology is a neoteric technology that transforms large molecules into nano-sized particles 1 -100 nanometers[4]. There are several nanoparticles that differ in their sizes, shapes, surface area, and function, as well as metal nanoparticles and metal oxides are of great importance due to their specialized qualities in fighting microbial communities[5].

Currently, titanium dioxide (TiO2) has become the focus of researchers because in addition to being approved by the Food and Drug Administration (FDA), it has great biological effects in environmental purification, pharmaceutical applications, solar energy cells as well as its catalytic ability Photosynthesis [6].

There are various methods for the synthesis of titanium dioxide nanoparticles such as physical methods, chemical methods and biological methods. Recently, biological methods are considered the most suitable methods because they are environmentally friendly and have low cost compared to other methods[7].

Pseudomonas aeruginosa is an opportunistic, non-lactose fermenter, gram-negative bacterium that is resistant to many antibiotics and produces many pigments, including Pyocyanin, a low molecular weight bluish-green pigment that is one of the most important virulence factors of bacteria and acts as a reducing agent[8].

The main aim of the present study is to prove the effectiveness of biosynthetic Titanium dioxide nanoparticles on inhibiting the production of some virulence factors and curing the genes responsible for their encoding in mice infected with *E. histolytica.*

Materials and Methods

Twenty-five fecal samples were collected for children infected with *E.histolytica* from the patients of Al Alalwia Children's Teaching Hospital and Central Child Hospital in Baghdad-Iraq who were suffering from moderate to severe diarrhea. Shield (Fecal Collection tube) used to preserve parasite genetic material for DNA extraction by using Quick-DNA™ Fecal/ Soil Microbe Miniprep Kit /Zymo/ USA and conducting a polymerase chain reaction (PCR) test using four pairs of primers from (Integrated DNA Technologies company, IDT (Canada) to detect the presence of the virulence factors Active cysteine proteinase, Amoebapore C, Lectin (hg13), Phospholipase of the parasite, as they were molecularly detected to confirm their presence in fecal samples.

Specific primers of virulence factors:-

Table 1. The specific primer of gene Cysteine proteinase

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward Primer	5′- TTTCAATACTTGGGTTGCAAAT -3′	51.3	31.8 %	885 bp base pair (11)
Reverse Primer	5′ GCAGCTCCTGAAGCAATACC-3′	56.2	55 %	

GenBank accession number X91644

2024, 21(3): 0850-0866
https://dx.doi.org/10.21123/bsj.2023.7205
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Table 2. The specific primer of gene Amoebapore C

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward Primer	5′- AAGGTAAATTGAATCAAAACACAAA -3′	50.4	24%	928bp base pair (12)
Reverse Primer	5′ TCGACCGTTTGTTTACTTCTCA-3′	54.1	40.9 %	

Table 3. The specific primer of gene Lectin (hg13)

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward Primer	5′- GACATATGCAACAAAAACTGAAGC - 3′	53.4	37.5 %	900bp base pair
Reverse Primer	5′ ACACTTGGTTTTCACTTTACGT-3′	52.8	36.4 %	(11)

Genbank accession number L14815 and TIGR genome database loci 29.m00206 and 290.m00068

Table 4. The specific primer of gene phospholipase

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward Primer	5′- TGCTGATTTGGCTCTTGGGA -3′	56.9	50%	420bp base pair
Reverse Primer	5′ CCAAGCCCTCTTTCCCCAAA-3′	57.8	55%	(12)

Thirty *Musmusculus* mice were selected and divided into six groups that included the first group (negative control group), and the amoebic suspension was administered orally to five groups of laboratory mice, and the infection was confirmed both microscopically and molecularly. Biosynthesis of Titanium dioxide nanoparticles Titanium dioxide nanoparticles was synthesized from *P.aeruginosa*producing Pyocyanin dye as a reducing agent to form Titanium dioxide nanoparticles. The biosynthetic titanium dioxide was characterized using several tests including: X-ray diffraction, field emission scanning electron microscope (FE-SEM), Fourier transform infrared spectroscopy (FTIR), UV visible spectroscopy, Atomic force microscope (AFM). The average diameter of the Titanium dioxide particles was 59.69 nanometers.

After confirming the presence of infection, 5 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. As for the remaining 20 mice, they were divided into four groups that were treated by intraperitoneal injection. Intraperitoneal injection (IP) with four different concentrations of biosynthetic Titanium dioxide nanoparticles 2, 3, 4, 6 mg/ Kg to determine the effect of titanium dioxide nanoparticles in inhibiting the production of virulence factors[9].

2024, 21(3): 0850-0866
https://dx.doi.org/10.21123/bsj.2023.7205
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Results and Discussion

Results of detection of virulence factors of *E. histolytica* samples.

Active Cysteine proteinase

The results of the detection of the virulence factor Cysteine proteinase showed its presence in 11 samples, or only 44%, as shown in (Fig .1) and (Table 5).

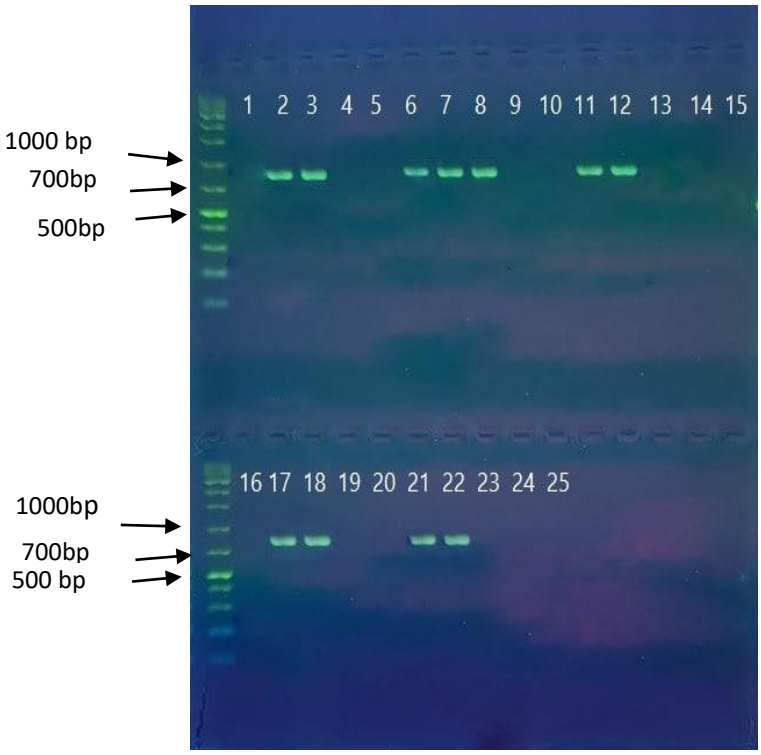

Figure 1. PCR product of Cysteine proteinase for *E. histolytica*,the band size 855 bp , the product was electrophoresis on 2% agarose , 70 Vol, 60 amp, 1x TBE buffer for 1.5 h, ladder 1000 bp.

Cysteine proteinase, which is the most important virulence factor secreted by the parasite, dissolves host tissues, kills host cells in contact with it, induces apoptosis of target host cells and through this mechanism distinguishes the Trophozoite of pathological species of the genus Amoeba such as *E. histolytica* differs from other non-pathological species and this is agree with [10] who demonstrated the role of Cysteine proteinase and its essential role in the interactions between parasite and host, including nutrient acquisition, facilitating tissue invasion and defense against body immunity.

Amoebapore C

Virulence factor results of the detection of Amoebapore C showed its presence in only 12 samples, as shown in (Fig. 2 and Table 5).

2024, 21(3): 0850-0866
https://dx.doi.org/10.21123/bsj.2023.7205
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

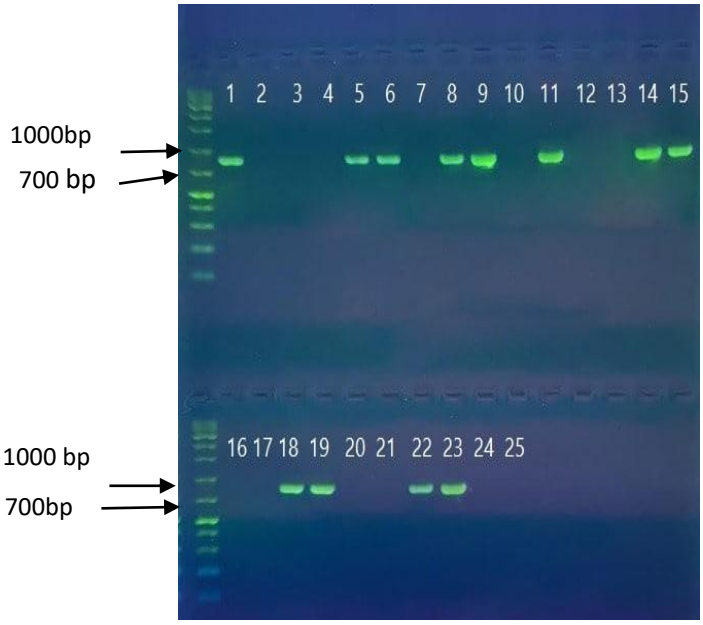

Figure 2. PCR product of Amoebapore C for *E.histolytica*. band size 928 bp, the product was electrophoresis on agarose 2%, 70 Vol, 60 amp, 1x TBE buffer for 1.5 h, ladder 1000 bp.

The virulence factor Amoebapore C plays a very important role in bloody diarrhea because it causes tissue breakdown and amoebic colitis. The results of this study showed its presence in 52% of the examined fecal samples, and these results agree with [11] they found that Only 58 samples were positive for Ameobapore C, while they do not agree with[12], which found that the above virulence factor is present in all samples. Studies have shown that trophozoite that lacks Amoabapore C is less virulent [13].

Lectin (hg13)

The results of the detection of the virulence factor Lectin (hg13) showed its presence in only 13 samples, as shown in (Fig. 3 and Table 5).

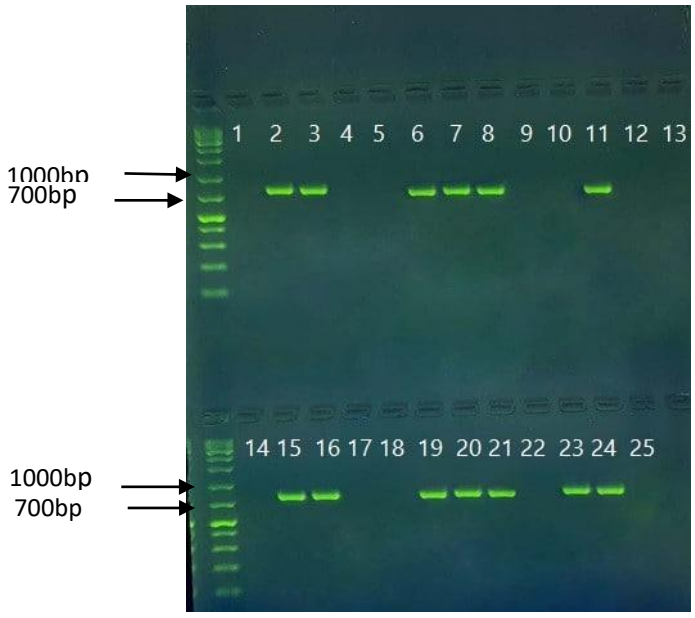

Figure 3. PCR product for Lectin (hg13) for *E. histolytica*, band size 900 bp, the product was electrophoresis on agarose gel 2%, 70 Vol, 60 amp, 1x TBE or buffer for 1.5 h, ladder 1000 bp.

2024, 21(3): 0850-0866
https://dx.doi.org/10.21123/bsj.2023.7205
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

The virulence factor is GAL/ NaG Lectin, as the results of this study showed that its presence was 52%, and these results are agreeing with [11] who found that the above virulence factor is present in 55% of the samples, as well as compatible with [14] who demonstrated that the adhesion of trophozoite to the intestinal mucosa is mediated by GAL/ /NaG Lectin, which is the first step in pathogenesis.

Phospholipase

The results of the detection of the virulence factor Phospholipase showed its presence in only 12 samples, as shown in (Fig. 4 and Table 5).

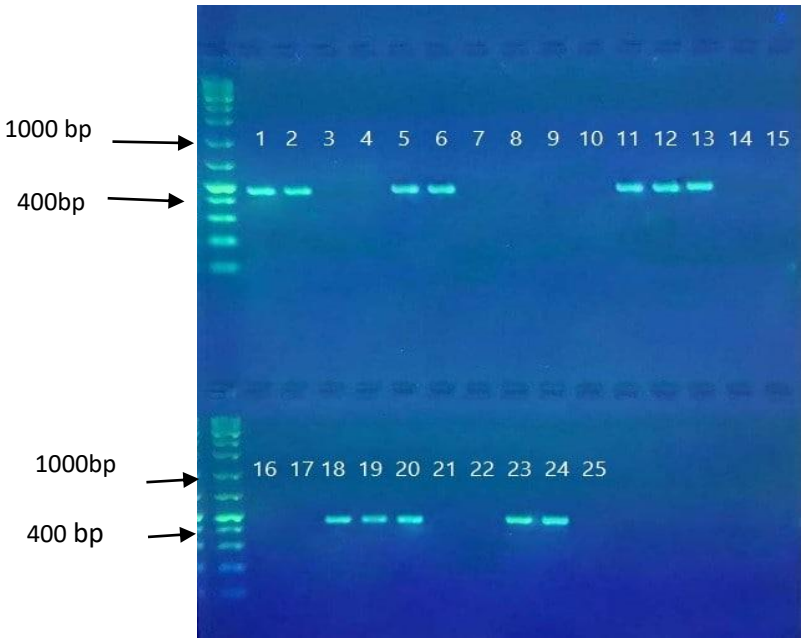

Figure 4. PCR product of Phospholipase for *E.histolytica*, band size 420 bp, the product was electrophoresis on agarose gel 2%, 70 Vol, 60 amp, 1x TBE or buffer for 1.5 h, ladder 1000 bp.

Through the detection of parasite virulence factors genes in excrement samples, it was found that the four virulence factors genes are present in samples No 6 and No 11. The two samples were taken to infect laboratory animal.

Table 5. The genes of the four virulence factors in the parasite isolates before the experiment *in vivo*

PCR EH	*Cysteine proteinase*	Lectin (hg13)	*Amoebapore C*	*Phospholipase*	sample ID
+	-	-	+	+	1
+	+	+	-	+	2
+	+	+	-	-	3
+	-	-	-	-	4
+	-	-	+	+	5
+	+	+	+	+	6
+	+	+	-	-	7
+	+	+	+	-	8
+	-	-	+	-	9

2024, 21(3): 0850-0866
https://dx.doi.org/10.21123/bsj.2023.7205
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

+	-	-	-	-	10
+	+	+	+	+	11
+	+	-	-	+	12
+	-	-	-	+	13
+	-	-	+	-	14
+	-	+	+	-	15
+	-	+	-	-	16
+	+	-	-	-	17
+	+	-	+	+	18
+	-	+	+	+	19
+	-	+	-	+	20
+	+	+	-	-	21
+	+	-	+	-	22
+	_	+	+	+	23
+	_	+	-	+	24
+	-	-	-	-	25

Statistical analysis was carried out using chi-square to find out the percentage of virulence factors of *E. histolytica* in the fecal samples as shown in Table 6, and it was proved that there were no significant differences in the presence of virulence factors in 25 fecal samples, as the value of Chi-Square was 0.321 and the P-Value 0.956, Significance level $P \leq 0.05$.

Table 6. Statistical analysis for the detection of the genes of the four virulence factors

Groups	+VE	%	-VE	%	Total %
Cysteine proteinase	11	44.0	14	46.0	%100
Lectin (hg13)	13	52.0	12	48.0	%100
Amoebapore C	12	48.0	13	52.0	%100
Phospholipase	12	48.0	13	52.0	%100
Total	48		52		
	Ns				
	Chi-Square = 0.321 P-Value = 0.956				
	Significance level ($P \leq 0.05$)				

2024, 21(3): 0850-0866
https://dx.doi.org/10.21123/bsj.2023.7205
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Active Cysteine proteinase

The results of molecular detection of the effect of TiO_2NPs in inhibiting the ability of the parasite to produce the virulence factor Cysteine proteinase by curing the gene responsible for encoding it showed that 2, 3 mg/Kg of TiO_2NPs did not affect the production of the virulence factor when treated with the third to the tenth day of infection, as shown (Fig. 5 and Table 7). While, the doses of Titanium dioxide nanoparticles 4, 6 mg/Kg showed their ability to inhibit the parasite's ability to produce Cysteine proteinase and curing the gene responsible for encoding it from the third to the tenth day of treatment, which indicates the effectiveness of these doses and since the first use on the third day of infection, and when Conducting the statistical analysis using Dunkin' polynomial test, it was found that there were significant differences between the doses of Titanium dioxide nanoparticles 2 ,3 and 4,6 mg/Kg in inhibiting the parasite's ability to produce virulence factor and the days of treatment as shown in Table 8.

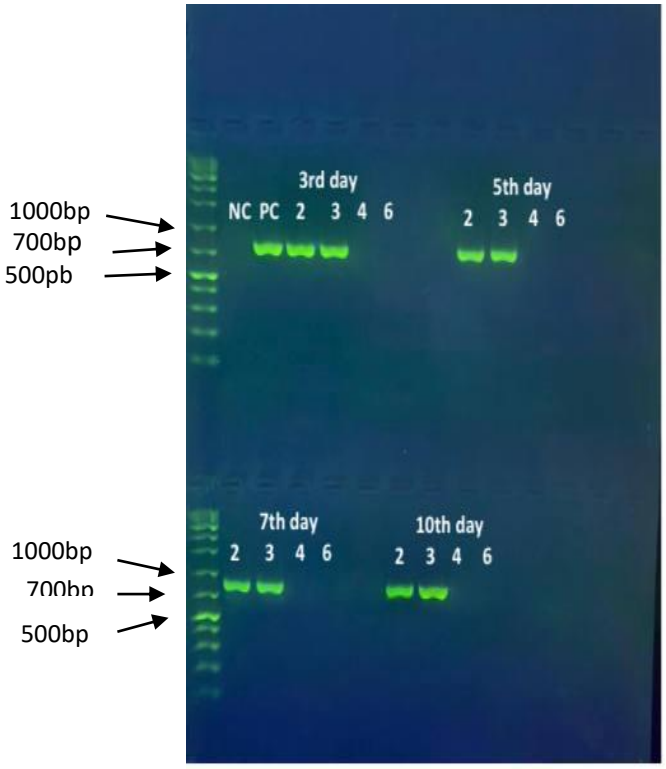

Figure 5. PCR product electrophoresis to detect the gene encoding Cysteine proteinase of *E. histolytica*, 855 bp band size, agarose gel 2%, 70 Vol, 60 amp 1.5 h, ladder 1000 bp.

Table 7. Presence of the gene responsible for the virulence factor Cysteine proteinase after treatment with TiO2 NPs

First line

Ladder	NC	PC	dose (mg/Kg)							
			2	3	4	6	2	3	4	6
	-	+	+	+	-	-	+	+	-	-
			Treatment on third day				Treatment on fifth day			

2024, 21(3): 0850-0866
https://dx.doi.org/10.21123/bsj.2023.7205
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Second line

Ladder	dose (mg/Kg)							
	2	3	4	6	2	3	4	6
	+	+	-	-	+	+	-	-
	Treatment on seventh day				Treatment on tenth day			

Table 8. Effect of Titanium dioxide nanoparticles with different concentrations on the ability of *E. histolytica* to produce the virulence factor cysteine proteinase.

Days of treatment	Titanium dioxide dose (mg/Kg)				The number of virulence factor produced according to the number of days after treatment
	2	3	4	6	+VE
the third day	1	1	0	0	2 a
the fifth day	1	1	0	0	2 a
the seventh day	1	1	0	0	2 a
the tenth day	1	1	0	0	2 a
Total virulence by Dose	4 a	4 a	0 B	0 b	

*Similar letters refer to that there are no significant differences between Titanium dioxide nanoparticles concentrations and treatment days

*Different letters refer to significant differences between Titanium dioxide nanoparticle dose and treatment days

*N.C refers to the negative control group (uninfected mice).

*P.C refers to the positive control group (infected mice untreated with Titanium dioxide nanoparticles)

Virulence factor Amoebapore C

The results of the molecular detection showed difference in the effect of Titanium dioxide nanoparticles on the ability of the parasite to produce the virulence factor under study, as all dose of TiO_2NPs did not affect the ability of the parasite to produce the virulence factor Amoebapore C upon treatment on the third day of infection, as shown in Fig. 6 and Table 9. Also, the concentrations 2,3 mg/Kg on the fifth, seventh and tenth days also did not affect the ability of the parasite to produce the virulence factor Amoebapore C. Whereas, the concentrations of 4 ,6 mg/Kg showed inhibition in the ability of the parasite to produce the virulence factor by curing the gene responsible for encoding it, and when statistical analysis was carried out using Dunkin' polynomial test, significant differences were found between the concentrations of Titanium dioxide nanoparticles 2,3 and 4,6 mg/Kg inhibiting the ability of the parasite to produce virulence factor and the days of treatment, as shown in Table 10.

2024, 21(3): 0850-0866
https://dx.doi.org/10.21123/bsj.2023.7205
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

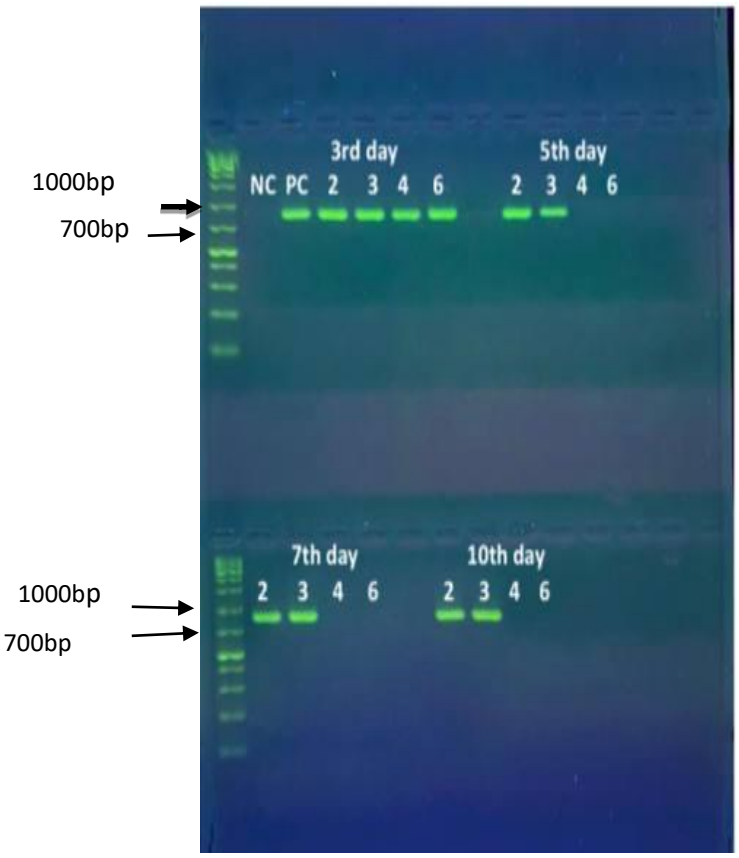

Figure 6. PCR product electrophoresis to detect of the gene encoding Amoebapore C of.*E. histolytica*, band size 928 bp, acrose gel 2%, 70 Vol, 60 amp, 1x TBE buffer for 1.5 h, ladder 1000 bp.

Table 9. Presence of the gene responsible for the virulence factor Cysteine proteinase after treatment with TiO2 NPs

First line

Ladder	NC	PC	dose (mg/Kg)							
			2	3	4	6	2	3	4	6
	-	+	+	+	+	+	+	+	-	-
			Treatment on third day				Treatment on fifth day			

Second line

Ladder	dose (mg/Kg)							
	2	3	4	6	2	3	4	6
	+	+	-	-	+	+	-	-
	Treatment on seventh day				Treatment on tenth day			

2024, 21(3): 0850-0866
https://dx.doi.org/10.21123/bsj.2023.7205
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Table 10. Effect of Titanium dioxide nanoparticles with different concentrations on the ability of *E. histolytica* to produce the virulence factor Amoebapore C.

Days of treatment	Titanium dioxide dose mg/Kg				The number of virulence factor produced according to the number of days after treatment
	2	3	4	6	+VE
the third day	1	1	1	1	4 a
the fifth day	1	1	0	0	2 b
the seventh day	1	1	0	0	2 b
the tenth day	1	1	0	0	2 b
Total virulence by dose	4	4	1	1	
	A	a	b	b	

Virulence factor lectin (hg13)

The results of the molecular detection showed difference in the effect of ofTiO_2NPs on the ability of the parasite to produce the virulence factor under study, that the dose 2, 3 mg/Kg of TiO_2NPs did not affect the ability of the parasite to produce the virulence factor lectin (hg13). During treatment from the third to the tenth day of infection, as shown in Fig. 7 and Table 11.

For instance for the dose 2,3 mg/Kg ofTiO_2NPs, they were characterized by their ability to inhibit the virulence factor Lectin (hg13) and curing the gene responsible for its encoding from the third to the tenth day of treatment, which indicates the effectiveness of these concentrations and since the first use on the third day , When conducting statistical analysis using Dunkin's multinomial test, it was found that there were significant differences between the inhibitory effect of 2,3 and 4,6 mg/Kg of TiO_2NPs on the parasite's ability to produce virulence factor and treatment days, as shown in Table 12.

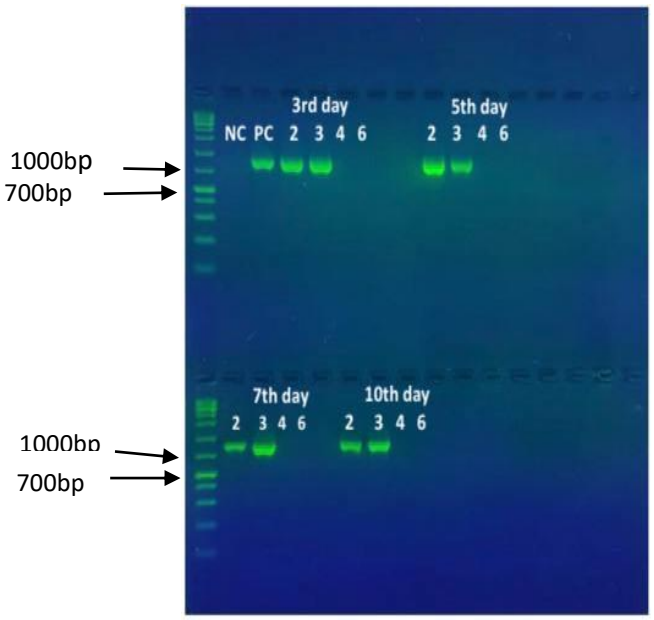

Figure 7. PCR product electrophoresis to detect of the gene encoding Lectin (hg13) of. *E. histolytica*, band size 900bp, agarose gel 2%, 70 Vol, 60 amp, 1x TBE buffer for 1.5 h, ladder 1000 bp.

2024, 21(3): 0850-0866
https://dx.doi.org/10.21123/bsj.2023.7205
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Table 11. Presence of the gene responsible for encoding the virulence factor Lectin (hg13) after treatment with Titanium dioxide nanoparticles.

First line

ladder	NC	PC	dose (mg/Kg)							
			2	3	4	6	2	3	4	6
	-	+	+	+	-	-	+	+	-	-
			Treatment on third day				Treatment on fifth day			

Second line

ladder	dose (mg/Kg)							
	2	3	4	6	2	3	4	6
	+	+	-	-	+	+	-	-
	Treatment on seventh day				Treatment on tenth day			

Table 12. The effect of Titanium dioxide nanoparticles with different concentrations on the ability of *E. histolytica* to produce the virulence factor Lectin (hg13)

Days of treatment	Titanium dioxide dose mg/Kg				The number of virulence factor produced according to the number of days after treatment
	2	3	4	6	+VE
the third day	1	1	0	0	2 a
the fifth day	1	1	0	0	2 a
the seventh day	1	1	0	0	2 a
the tenth day	1	1	0	0	2 a
Total virulence by dose	4	4	0	0	
	a	a	B	b	

Virulence factor Phospholipase

The results of treatment of infection with *E. histolytica* parasite with nanoparticles of titanium dioxide showed that the four concentrations during the different treatment days did not affect the ability of the parasite to produce the virulence factor Phospholipase of *E .histolytica* as shown in Fig. 8 and Table 13. Also, when performing the statistical analysis, no significant differences appeared using the four concentrations of biosynthetic Titanium Dioxide Nanoparticles during the different treatment days as shown in Table 14.

2024, 21(3): 0850-0866
https://dx.doi.org/10.21123/bsj.2023.7205
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

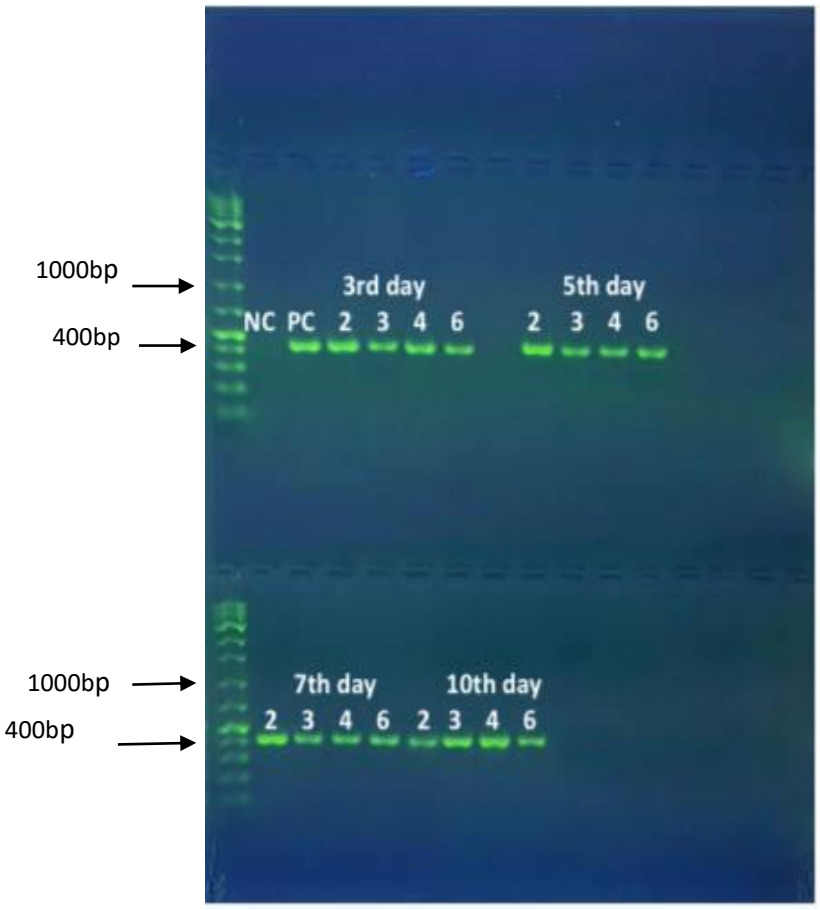

Figure 8. PCR product electrophoresis to detect of the gene encoding Phospholipase of. *E. histolytica*, band size 420bp, agarose gel 2%, 70 Vol, 60 amp, 1x TBE buffer for 1.5 h, ladder 1000 bp.

Table 13. Presence of the gene responsible for encoding the virulence factor phospholipase after treatment with Titanium dioxide nanoparticles.

First line

Ladder	NC	PC	dose (mg/Kg)							
			2	3	4	6	2	3	4	6
	-	+	+	+	+	+	+	+	+	+
			Treatment on the third day				Treatment on the fifth day			

Second line

Ladder	dose (mg/Kg)							
	2	3	4	6	2	3	4	6
	+	+	+	+	+	+	+	+
	Treatment on the seventh day				Treatment on the tenth day			

2024, 21(3): 0850-0866
https://dx.doi.org/10.21123/bsj.2023.7205
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

Table 14. Effect of Titanium dioxide nanoparticles with different concentrations on the ability of *E. histolytica* to produce phospholipase.

Days of treatment	Titanium dioxide dose (mg/ Kg)				The number of virulence factor produced according to the number of days after treatment
	2	3	4	6	+VE
the third day	1	1	1	1	4 a
the fifth day	1	1	1	1	4a
the seventh day	1	1	1	1	4a
the tenth day	1	1	1	1	4a
Total virulence by dose	4	4	4	4	
	a	a	a	a	

This study is new of its kind in the synthesis of nanoparticles and their use in inhibiting the genes which are responsible of encoding virulence factors of *E. histolytica*, and there are studies similar to it to a certain extent, as[9] used commercially manufactured silver nanoparticles to determine their toxicity and *invitro* effect on the trophozoite of *E. hisolytica*, as it noticed a significant difference in the decrease in the number of trophozoites after incubation with silver nanoparticles, as dose 50,75 and 100 µg /ml were used, but the study was *in vitro*.

The results of this study are similar to [15] as the results of their use of copper oxide nanoparticles and silver nanoparticles, respectively, showed a significant effect of reduction the cysts vitality in *E. histolytica* and *Cryptosporidium parvum.* These nanoparticles were considered as safe and effective therapeutic alternatives against Parasites above. The studies also demonstrated the effectiveness of gold and silver nanoparticles, as well as metal oxides, that have an inhibitory ability and were considered antiparasitic against different types of parasites Giardia, Plasmodium, and Toxoplasma[16]. Likewise, the results of this study in the use of nanoparticles as antiparasitic agents agreed with the results of the study reached by [17] which showed that Selenium and copper oxide nanoparticles were used to determine the extent of their impact on the number of cysts of the *Giardia duodenalis*.

The study was conducted by *in vitro* and nanoparticles have their unique physical and chemical properties, and because they are small in size, they have a large surface area, electric charge, and a distinctive shape [18] Its surface area interferes with various biological molecules and microorganisms and destroys the negative activity of parasites and can enter cells more than other molecules[19]. For instance the nanoparticles interfere with the surface of the parasite and cause damage and destruction to the molecules of Lipophosphoglygan and Glygoprotein that are present on the surface of the parasite and responsible for infection, and that these molecules are affected by the Reactive Oxygen Species (ROS) generated by the nanoparticles and this leads to the failure of infection with the parasite[20].

Conclusion

The results of the study showed the ability of TiO_2NPs as antiparasitic of TiO_2NPs to inhibit virulence factors production of parasite and curing the genes which are responsible for coding them when using dose (4, 6) mg/Kg of TiO_2NPs thus reducing the pathogenicity of *E. histolytica* and statistical analysis showed significant differences between the use of TiO_2NPs and the days of treatment.

Acknowledgment

We would like to thank medical staff in Al Alawia Children's Teaching Hospital and Central Child Hospital in Baghdad for their outstanding role in collecting data for this study.

Authors' Declaration

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are ours. Furthermore, any Figures and images, that are not ours, have been included with the necessary permission for re-publication, which is attached to the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Kirkuk.

Authors' Contribution Statement

L. T. Y. L. contributed to the experiment working and writing the article. Sh. A. A. and M. N. M. designed the methodology and reviewed the article.

References

1. Al-Areeqi MA, Sady H, Al-Mekhlafi HM, Anuar TS, Al-Adhroey AH, AtrooshWM, et al. First molecular epidemiology of Entamoebahistolytica, E. dispar and E. moshkovskii infections in Yemen: different species-specific associated risk factors. Trop Med Int Health. 2017; 22(4): 493–504 https://doi.org/10.1111/tmi.12848
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 . 2019; PloS One. 14(2): 0212791 https://doi.org/10.1371/journal.pone.0212791
3. Rostami NM,Dabiri H,Zali MR,Kazemi B,Haghighi A. Discrimination of Entamoebamoshkovskii in patients with gastrointestinal disorders by single-round PCR. Jpn J Infect Dis. 2010; 63(2): 136-138. https://doi.org/10.7883/yoken.63.136
4. Dos Santos RMA,Da Silva PB,Spósito L, De Toledo LG, Bonifácio BV, RoderoCF,Dos Santos KC, Chorilli M,Bauab TM. Nanotechnology-based drug delivery systems for control of microbial biofilms: a review. Int J Nanomedicine. 2018 ; 13:1179-1213. https://doi.org/10.2147/IJN.S146195
5. Luaibi NM , Raghda Ali Mohammed RA. Physiological and Hormonal Effects of Titanium Dioxide Nanoparticles on Thyroid Function and the Impact on Bodyweight in Male Rats. Baghdad Sci J. 2022: 416-424. http://dx.doi.org/10.21123/bsj.2022.3683
6. MishraV, Sharma R,DutJasuja.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. https://doi.org/10.2147/IJN.S200254
7. Nithya R,RagunathanR..Synthesis of silver nanoparticle using Pleurotussajorcaju and its antimicrobial study. Dig J Nanomater Bios. 2009; 4(4): 623-629. https://doi.org/10.2147/IJN.S52306
8. DeBritto S,Gajbar TD,Satapute P,Sundaram L,Lakshmikantha RY. Jogaiah S. et al. Isolation and characterization of nutrient dependent pyocyanin from Pseudomonas aeruginosa and its dye and agrochemical properties. Sci Rep. 2020; 10: 1542 https://doi.org/10.1038/s41598-020-58335-6.
9. Ahmed ZA, Mustafa TH A,Ardalan NM,Idan EM . In Vitro Toxicity Evaluation of Silver Nanoparticles on Entamoeba histolytica trophozoite. Baghdad Sci J. 2017; 14(3): 509-515. https://doi.org/10.21123/bsj.2017.14.3.0509
10. Rostami S, Rezaeian M, Jamali R,Rezaie S, Babaei Z, Hooshyar H. Differences in Entamoebahistolytica Cysteine Proteinase 5 Gene Isolated From Bandar Abbas and Tabriz, Iran Int J Enteric Pathog. 2017; 5(2): 49-53. https://doi.org/10.15171/ijep.2017.12
11. Al-DamerchiATN, Al-Ebrahimi HN. Detection of major virulence factor of Entamoeba histolytica by using PCR technique. AL- Qadisiya. Med J. 2016; 12(21): 36-45. https://doi.org/10.22092/ARI.2022.359133.2375
12. Al-KaeebiSRA, Al-Difaie RSS.. Use PCR conventional for detecting AP and PLA virulence factors of Entamoeba histolytica in patients stool samples in Al-Qadisiyah Province. Wasit j Sci Med. 2016; 8(4): 102- 110.

13. AndraJ,Herbst R,Leippe M. Amoebapores, archaic effectors peptides of protozoan origin, are discharged into phagosomes and kill bacteria by permeabilizing their membranes. Dev Comp Immunol. 2003; 27: 291–304. https://doi.org/10.1016/s0145-305x(02)00106-4
14. Petri WA, Haque R, Mann BJ. The bittersweet interface of parasite and host: Lectin-carbohydrate interactions during human invasion by the parasite Entamoebahistolytica. Annu Rev Microbiol. 2002; 56: 39–64. https://doi.org/10.1146/annurev.micro.56.012302.160959
15. Saad A HA, Soliman MI, Azzam A M, MO AB. Antiparasitic activity of silver and copper oxide nanoparticles against Entamoebahistolytica and Cryptosporidium parvum cyst. J Egypt Soc Parasitol. 2015; 45(3): 593-602. https://doi.org/10.12816/0017920
16. ElmiT ,Gholami S,Fakhar M, Azizi F. A Review on the use of nanoparticles in the treatment of parasitic infections. J. Mazand. Univ. Med. Sci. 2013; 23(102):126-33.
17. Malekifard F,Tavassoli M,Vaziri K.In Vitro Assessment Antiparasitic Effect of Selenium and Copper Nanoparticles on Giardia deodenalis. Cyst Iran J Parasitol. 2020; 15(3): 411–417. https://doi.org/10.18502/ijpa.v15i3.4206
18. Wang H, Wick RL, Xing B. Toxicity of nano-particulate and bulk ZnO, Al2O3 and TiO2 to the nematode Caenorhabditiselegans. Environ Pollut. 2009; 157(4): 1171–7. https://doi.org/10.1016/j.envpol.2008.11.004
19. Mahmoudvand H, Shakibaie M, Tavakoli R, Jahanbakhsh Sareh, Sharif Iraj. In vitro study of leishmanicidal activity of biogenic selenium nanoparticles against Iranian isolate of sensitive and glucantime-resistant Leishmaniatropica. Iran J Parasitol. 2014; 9(4): 452–460. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4345083/
20. Choi O, Hu Z. Size dependent and reactive oxygen species related nanosilver toxicity to nitrifying bacteria. Environ Sci Technol. 2008; 42(12): 4583-8. https://doi.org/10.1021/es703238h

2024, 21(3): 0850-0866
https://dx.doi.org/10.21123/bsj.2023.7205
P-ISSN: 2078-8665 - E-ISSN: 2411-7986
Baghdad Science Journal

الكشف جزيئيا" عن قدرة جزيئات ثنائي أوكسيد التيتانيوم النانوية المخلقة حيويا" في تحييد بعض جينات عوامل ضراوة *Entamoeba histolytica*

ليما طارق يوأش لازار[1]، شهاب احمد الجبوري[2]، محمد نظير معروف[3]

[1]فرع العلوم التمريضية الاساسية، كلية التمريض، جامعة كركوك، كركوك، العراق.
[2]قسم علوم الحياة، كلية التربية للعلوم الصرفة، جامعة تكريت، تكريت، العراق.
[3]كلية الطب، جامعة تكريت، تكريت، العراق.

الخلاصة

تضمنت الدراسة الحالية تشخيص الطفيلي *Entamoeba histolytica* مجهريا" وجزيئيا" في عينات الغائط باستخدام بادئات متخصصة للكشف عن اربعة عوامل ضراوة يمتلكها الطفيلي شملت Active cysteine proteinase، N-acetyl- / Galactose D-galactose – lectin، Amoebapore C، Phospholipase. تم تخليق جزيئات ثنائي اوكسيد التيتانيوم النانوية TiO_2NPs من بكتريا *Pseudomonas aeruginosa* المنتجة لصبغة Pyocyanin كعامل مختزل لتكوين TiO_2NPs. بعد تخليق ثنائي اوكسيد التيتانيوم النانوي TiO_2NPs تم معرفة قدرته في تثبيط عوامل ضراوة الطفيلي وتثبيط الجينات المسؤولة عن تشفيرها وذلك بأستخدام اربعة جرعات مختلفة (2، 3، 4 ، 6) ملغم/ كغم واعطائها عن طريق الحقن تحت الغشاء البريتوني للحيوانات المختبرية المخمجة بالطفيلي وتم التأكد من الخمج جزيئيا" ثم المعالجة ولمدة عشرة ايام، اذ تم اعطائها في اليوم الثالث، الخامس، السابع، العاشر.اظهرت نتائج الدراسة عدم قدرة جرعات TiO_2NPs(2، 3) ملغم/ كغم من TiO_2NPs في تحييد الجين المسؤول عن Active cysteine proteinase, Gal/Gal Nac-lectinعند العلاج في اليوم الثالث والى اليوم العاشربل تمكنت الجرعات (4، 6) ملغم/ كغم ومنذ المعالجة في اليوم الثالث من الخمج وحتى اليوم العاشر من تثبيط الجين المسؤول عن عاملي الضراوة، وعند اجراء التحليل الاحصائي تبين وجود فروق معنوية بين استخدام التراكيز المختلفة من TiO_2NPs وايام العلاج. اما قدرة المادة النانوية المستخدمة في تحييد الجينات المسؤولة عن Amoebapore C فقد تبين ان التراكيز الاربعة لم تؤثر على تثبيط عامل الضراوة عند العلاج في اليوم الثالث من الخمج. اما عند العلاج في اليوم الخامس والسابع والعاشر فأن الجرعات (2، 3) ملغم/كغم لم تتمكن من تثبيط Amoebapore C بينما الجرعات (4، 6) ملغم/ كغم تمكنت من تثبيط الجين المسؤول عن تشفيره وعند اجراء التحليل الاحصائي تبين وجود فروق معنوية بين استخدام لهذه المادة وايام العلاج.أخيراً فقد اظهرت نتائج الدراسة بأن الجرعات الاربعة لم تتمكن من تثبيط Phospholipaseمنذ اليوم الثالث وحتى اليوم العاشر، وعند اجراء التحليل الاحصائي تبين عدم وجود فروق معنوية.

الكلمات المفتاحية: تحييد الجينات، Entamoeba histolytica، صبغة البايوسيانين، Pseudomonas aeruginosa، جزيئات ثنائي اوكسيد التيتانيوم النانوية، ،عوامل الضراوة.