Effect of Titanium Dioxide Nanoparticles (TiO2NPs) on Leishmania parasite in vitro

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Abstract— The study included detecting the effect of nanoparticles of titanium dioxide nanoparticles (TiO2NPs) with different concentrations (0.5, 1, 5, 10) mg/ml and for two sizes (100, 10) nm on parasites of Leishmania tropica in vitro. The results showed that titanium dioxide nanoparticles (TiO2NPs) have an effect on the parasite with an inverse relationship between growth rate, concentrations of nanoparticles and the exposure period as well as the size of the nanoparticles. The sizes used of titanium dioxide nanoparticles (TiO2NPs) with all their concentrations had a toxic effect on Leishmania promastigote developing in culture media at 48 and 72 hours compared to the control group and that the concentrations of size 10 nm were more effective than concentrations of size of 100 nm on the growth of the parasite during the same time peroids used, and this gives an indication of the importance of using small sizes of titanium dioxide nanoparticles (TiO2NPs) as an anti-growth of Leishmania parasites.

Keywords—leishmania tropica, titanium dioxide nanoparticles, leishmania parasite

1 Introduction

Leishmania is one of the parasites that cause Leishmaniasis, and it is a neglected tropical disease according to the World Health Organization (WHO) and it is endemic to about 88 countries around the world. This disease was previously divided into three types: Cutaneous leishmaniasis (CL), visceral leishmaniasis (VL) and Mucocutaneous leishmaniasis (MCL). Currently the disease is divided into localized cutaneous leishmaniasis, diffuse cutaneous leishmaniasis, chronic cutaneous leishmaniasis, post kala-azar cutaneous leishmaniasis (PKDL), visceral leishmaniasis and mucocutaneous leishmaniasis [1]

Cutaneous leishmaniasis (CL) is a main public health problematic and reasons a variety of diseases from self-healing infections to prolonged disfiguring disease [2]. Recent antileishmanial chemotherapy applications are far away from being effective[3]. While pentavalent antimonial drugs are the most frequently prescribed treatments for leishmaniasis, they yield severe side effects [4]. Nanotechnology has performed as an attractive alternative due to its enhanced bioavailability and lower toxicity, and extra characteristics that advantage to reduce the danger of this disease [5][6].

The nanoparticles (NPs) are particles that have dimensions of (1-100) nanometers and they can be classified into different classes depending on their properties, shape

and sizes [7]. Nanotechnology involves finding molecules with precise specifications that work more efficiently than larger materials with the same chemical composition [8][9]. As the size of the particles plays a key role in it's antimicrobial activity, as small particles showed higher activity against microbes and parasites than large particles [10] and this is due to their penetration and entry into the cell [11], and There is a relationship between the antimicrobial properties and the total area of nanoparticles [12][13].

The use of nanobiotechnology has shown great progress in the treatment of parasitic infections as it's unique properties have inhibitory effects on

parasites [14][15], which made it used in medicine and in wide fields [16], so the aim of the current study is to show the effect of titanium dioxide nanoparticles (TiO2NPs) on Leishmania parasites in vitro.

2 Materials and methods

Parasite isolated

The *Leishmania tropica* strain was obtained from Al-Nahrain University.

2.1 Preparation of the culture medium

Toby's medium

Leishmania parasites were grow in the diphasic media prepared by [17]. The first is the solid phase containing blood agar and the second is the liquid phase. Table (1) shows the components of the medium.

Table 1. Solid medium

Seri	Ingredients	Amount
1	Meat extract	1.5 g
2	Biological peptone	2.5 g
3	Sodium chloride, Nacl	4.0 g
4	Agar	7.5 g
5	Distilled water	500 ml
6	Crystalline penicilline	200,000 IU
7	Streptomycin Sulfate	200 mg

The materials in solid medium are dissolved in distilled water except for antibiotics, then the pH is adjusted at 7.2-7.4 and sterilized with an Autoclave for 15 minutes under pressure 1 Atmosphere and a degree of 121° C. The solution is left to cool to approximately 45° C, then blood and antibiotics are added.

Table 2. liquid medium

Seri	ingredients	amount
1	Sodium chloride, Nacl	8.0 g

2	Polassium chloride , KC	0.2 g
3	Calcium chloride, $CaCl_2.6H_2o$	0.2 g
4	Potassium dihydrogen phosphate, KH_2PO_4	0.3 g
5	Glucose	2.5 g
6	Distilled water	1000 ml
7	Crystalline penicillin	200,000 IU
8	Streptomycin sulfate	200 mg

The above-mentioned contents in the table are dissolved in distilled water except for antibiotics, then the pH is adjusted at 7.2-7.4 and sterilized with an Autoclave for 15 minutes under pressure 1 Atmosphere and degree 121 ° C. Leave the solution to cool down and then add antibiotics.

2.2 Blood source

Human blood was used in all experiments related to the growth and preservation of Leishmania parasites.

2.3 Prepare the complete medium

A 15% of the inactivated blood is added at 56° C for period of 30 minutes to the basic medium (solid medium), then 5 ml distributed in 25 ml McCantry glass bottles and placed at an angle until it hardens. Then 2 ml of liquid medium is added to it when using.

2.4 Growing and counting the number of parasites

A 1.9 ml of the liquid medium was added to the 25 ml bottles containing the solid medium, then 0.1 ml of Leishmania promastigote, which was taken from the original cultures at the logarithmic phase of growth with an initial number of 2 x 105 parasites / ml, was added to it and then incubated at 25 ° C for 5 days.

The number of parasites is a counting by using a Haemocytometer slide by taking 0.5 or 1 ml of culture and fixing it with formalin 10%, one or two drops per ml. All these additions must be made in sterile conditions to prevent contamination and when the growth is high, they are diluted with physiological solution 0.9% [18].

2.5 Preparation of a solution of titanium dioxide nanoparticles (TiO2NPs)

Four concentrations of titanium dioxide nanoparticles solution were prepared according to [19] method, weighing (5, 10, 50, 100) mg of (TiO2NPs) from each size of titanium (10,100) nm and added to (10) ml of sterile distilled water, and then shaken by hand vigorously for (5-10) minutes to break the agglomeration and get a homogeneous solution and thus concentrations were (0.5, 1, 5, 10) mg/ml.

2.6 Determining the effect of titanium dioxide nanoparticles (TiO2NPs) on parasite's vitality

Concentrations of TiO2NPs (0.5, 1, 5, 10) mg/ml for each size (10 and 100) nm were used to determine the extent of its effect on the vitality and growth of Leishmania parasites, as these concentrations Individually added to sterile McCantry vials containing the aforementioned medium and compared with the control group with 3 replicates for each concentration.

The media used in the experiment were inoculated by adding 0.1 ml of the medium containing promastigote at the logarithmic phase with a number of 2 x 105 parasites / ml and incubated at 25 °C for 4 days. The number of parasites was counted on the second day 48 hours and the third day 72 hours of growth. The percentage of growth and finding the LD50 concentration at the logarithmic phase 72 hours.

2.7 Statistical analysis

The data were analyzed according to the CRD system of international trials, using Duncan's-Multiple-Range-Test. The significantly different averages were distinguished by different alphabetic letters.

3 Results

The results of the present study shows that TiO2NPs had an inhibitory effect against Leishmania promastigote, with an inverse relationship between growth rate, substance concentration, exposure period, as well as the size of the substance used. Table (3) shows that the highest concentration of growth inhibition for size 10 nm was 10 mg/ml during the time period 72 hours and that the lowest concentration of growth inhibition was 0.5 mg/ml during the time period of 48 hours, and that the concentration that lethal 50% of the parasites is (5) mg/ml.

Table 3. Shows the effect of several concentrations of titanium dioxide nanoparticles with a size of (10) nanometers on the growth of Leishmania promastigote (106×) compared to the control group.

Time(hour) / Concentration mg /ml	48 average	% For inhibition	72 average	% For inhibition
control	12.27 a	-	46.90 a	-
0.5	10.0 b	20	39.20 b	16

1	8.2 c	33	27.70 c	40
5	7.0 d	43	23.20 d	50
10	5.60 e	46	17.50 e	63

- Each number represents an average of three replicates.
- Numbers followed by different letters vertically indicate the presence of significant differences between them at the probability level ($P \leq 0.05$) according to Duncan's test.

While the results of the analysis in table (4) The highest concentration of growth inhibition for size 100 nm was 10 mg/ml during the time period 72 hours and that the lowest concentration of growth inhibition was 0.5 mg/ml during the time period of 48 hours and that the lethal concentration for nearly 50% of parasites is (5) mg/ml.

The results of the analysis showed that there were significant differences between the numbers of treated and untreated promastigote within the exposure periods.

Table 4. Shows the effect of the interaction between the size and concentration of TiO2NPs and treatment time on the growth of the Leishmania Promastigote (106 ×).

Size /Nano	Time (hours)	% of growth inhibition
10	48	8.61 c
	72	32.90 a
100	48	7.84 d
	72	30.84 b

1. Discussion

The results of the current study showed a significant difference in the effect between the sizes 10 and 100 nm in terms of the inhibitory activity of the growth of *leishmania* promastigote, as well as a significant difference in the effect between the percentage, size and treatment period and this is consisted with the results of many research and studies in terms of the inhibitory activity of titanium dioxide concentration and tested on many microorganisms, such as studying the effect of titanium dioxide nanoparticles in controlling secondary infection of *Echinococcus granulosus* parasite, where the concentrations were (1, 1.5, 3, 0.5, 0.2, 0.1, 0.05) mg/ml of (TiO_2NPs) effect on the rate of killing of protozoa [20] as well as studying the effect of Titanium Dioxide Nanoparticles (TiO_2NPs) on *Entamoeba gingivalis* was to increase the concentrations and the time factor has a positive effect on inhibiting the growth of the parasite and reducing the survival rate [21], and it agreed with our current study that there is an inverse relationship between the survival rate, the increase in concentration and the time factor.

The effect of titanium dioxide nanoparticles (TiO_2NPs) depends on the size of the particles, as the smaller the size, they accumulate in greater numbers on the surface of the cells, which causes oxidative stress and toxicity higher on cells [22]. Research has

shown the existence of killing mechanisms for living organisms on which nanoparticles work, including their ability to bind strongly between sulfur and phosphorous, which are compounds [23]. It contains sulfur and phosphorous in DNA or may accumulate significantly inside mitochondria and impair their function by oxidative stress [24][25]. Another mechanism of nanomaterials is the release of ions that contribute to cell killing by producing a high amount of ROS [26][27].

The mechanism of ROS generation within metal nanoparticles is a major mechanism of programmed cell death apoptosis that is caused by oxidative stress induced by nanoparticles [28][29], and since mitochondria are one of the major target organs of oxidative stress, high levels of nanoparticles can cause membrane phospholipids to break down and induce mitochondrial membrane depolarization [30]. Various metal oxides mediate ROS nanoparticles. for cell death by disruption of mitochondrial function [31][32].

4 Conclusions

The results of the current study showed that increasing the concentration of titanium dioxide nanoparticles (TiO_2NPs), the size of the material and the time factor had an inhibitory effect against the growth of *Leishmania* promastigote outside the body of the organism.

5 References

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Article submitted 16 January 2022. Published as resubmitted by the authors 25 June 2022.