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Original Article

Evaluation of the Efficacy of Sodium Chloride Nanoparticles on the Vitality of *Leishmania Major* (*in vitro*)

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Abstract

Leishmaniasis is one of the most important zoonotic diseases transmitted to humans by sand flies (*Phlebotomus spp*). *Leishmania major* promastigote causes Cutaneous Leishmaniasis in humans. The study aimed to investigate the effectiveness of Sodium Chloride nanoparticles (NaCl NPs) on the vitality of *Leishmania major* promastigote compared with the standard dose of Pentostam under laboratory conditions. Various concentrations of 2, 4, 6, and 8 µg/ml of the NaCl NPs were prepared. These concentrations were tested in vitro on *L. major* growth by the culture of the parasite in the cell culture microplate. After the fourth day, a different concentration of NaCl NPs was added with three replicates for each concentration. Later, the numbers of promastigotes were counted daily using a Haemocytometer stained by Trypan blue solution stain duration of the study, which continued for four days. The results showed that the Growth Index (GI) rate of *L. major* promastigote was decreased with increasing NaCl NPs concentration. The Growth Index rates were 1.32×10^6, 1.31×10^6, 0.95×10^6, and $0.78\times10^6$for the mentioned concentrations. These values were compared with the rate of the Pentostam group and control group, which were 1.09×10^6 and 3.43×10^6, respectively. The results revealed that the highest inhibition percentage was 92% for 8 µg/ml NaCl NPs after 96 hours, Pentostam group and control group, which were %86 and %0.00 for inhibition promastigote, respectively in the same period. The statistical analysis revealed a significant difference among concentrations at $P\leq0.05$ compared with the Pentostam and control groups. The current study concluded that the NaCl NPs have an excellent biological effect in inhibiting *L. major* promastigote growth in vitro. These promising results paved the way for employing NaCl NPs to treat human cutaneous leishmaniasis.

Keywords: Cutaneous leishmaniasis, *Leishmania major*, NaCl Nanoparticles, Pentostam

1. Introduction

Leishmaniasis is an important human disease caused by *Leishmania spp.*, a protozoan parasite belonging to the genus Leishmania. It is classified as Kinetoplastida, "Trypanosomatida" family in the old world, and "*Lutzomyia*genus" in the New World (1-3). Leishmaniasisisa vector-borne disease and estimated that more than 350 million people worldwide are at risk of the disease (4).

This parasite developed in the invertebrate host (final host) and then transmitted to the vertebrate host (intermediate host), such as humans, through the bite of infected sandflies (5-7). *Leishmania* spp. has two forms during its life cycle, promastigote and amastigote. The promastigote is represented in the infection stage, and detected in the female sandfly belonging to the genus *Phlebotomus* (the host-vector) in the Old World and the genus "*Lutzomyia"* in the new world (3, 8). Also, promastigote can be observable in processed cultural media (9).The second form is the Amastig ;this form is infected and replicated inside macrophage cells, positioned in the skin, mucous membranes, lymph

nodes, bone marrow, and spleen found in humans and reservoir hosts. This form infected and replicatedinside the macrophage, in the skin, mucous membranes, lymph nodes, bone marrow, and spleen (10, 11).

Leishmaniasis is a tropical disease that spreads widely in societies, especially in the Middle East, Asia (southwest and central Asia), Africa (in the tropics and North Africa), and southern Europe. It is also endemic to many Mediterranean countries (1). Estimated the prevalence of infected people with leishmaniasis is difficult, but some studies suggested about 12 million annual new infections, distributed over 98 countries (12, 13).

A wide range of clinical manifestations distinguishes leishmaniasis. It includes “cutaneous leishmaniasis” (CL), “mucocutaneous leishmaniasis” (MCL), and “visceral leishmaniasis” (VL) (6, 7). Cutaneous leishmaniasis represents 50-70% of all leishmaniasis cases. These cases have been reported from Afghanistan, Algeria, Pakistan, Saudi Arabia, Iran, Iraq, Peru, and Brazil (1).

One of its types was well known in Iraq and was called the Baghdad boil. Generally, there are two types of cutaneous leishmaniasis. The first type is “Zoonotic Cutaneous Leishmaniasis (ZCL)," when the parasite is transmitted from a group of animals to humans. The species *L. major* causes this type, and it appears in rural areas, and this type causes a moist ulcer. The second type is “Anthroponotic cutaneous Leishmaniasis" (ACL), the parasite is transmitted from person to person. The Ltropica species cause this type. It is the most common form in urban areas dry ulcers (dry sores) (6, 7). Both dry and wet cutaneous leishmaniasis were recorded in Iraq (5).

Today, leishmaniasis is treated with various methods. Aside from the sandfly’s insecticide resistance, the available drugs have side effects. The typical treatment against leishmaniasis depends on pentavalent Antimonial compounds like Pentostam (9, 14). However, many studies have discovered that Pentostam is toxic and has several side effects.There is an urgent need to find a new treatment for leishmaniasis (9). Therefore, the current study evaluated the efficacy of Sodium Chloride Nanoparticles (NaCl NPs) on L. *major* promastigote vitality as a save suggested treat which can be used in the future to reduce the spread of Cutaneous Leishmaniasis infection in humans.

2. Materials and Methods

2.1. Parasite Preparation and Culture

This isolation was cultured on RPMI-1640 medium, which, plus to it, fetal bovine serum FBS, with the value of 9 ml RPMI-1640+1 ml FBS. This medium was put in a conical test tube and then a 0.1 ml (parasitic density $1x10^6$ cell/0.1 ml). *L. major* isolation was added to cultured medium RPMI-1640. After that, all tubes were incubated at 25ºC and followed daily. After 96 hours (4 days), the color of the medium was transformed from pink to yellow. This color change was considered an indicator for promastigote growth (15).

2.2. Preparation of NaCl NPs

Sodium Chloride nanoparticles were equipped from Nano pars SPADANA. The compound features included 801ºC melting point, 1413ºC boiling point, colorless NaCl nanopowder, 99.9% purity, and soluble in an alcohol solution and high purity water.

The Initial concentration of NaCl NPs was 500 mg/ml.The stock solution was dispersed in high purity water using sonication with conditions as follows: 100 watts, 40 KHs/40 min. Small magnetic bars were added to suspension for motivation during mitigation to avoid aggregation. Four NaCl NPs concentrationsof 2, 4, 6, and 8$\mu g.ml^{-1}$ were prepared immediately from the stock solution (16).

2.3. Effect of NaCl NPs Concentrations on *L. major* Promastigote (*in vitro*)

A cell culture microplate was used to evaluate the effect of different NaCl NP concentrations on *L. major* promastigote viability. The experience was continuous for 24, 48, 72, and 96 hours. The culture of

promastigote is divided into three main groups: the pentostam group, control group, and experimental group. The experience (Figure 1) is designed as follows:

1. The control group contains 0.2ml culture of the parasite without any treatment (4 replications).

2. Pentostam group: contains 0.2 ml culture of the parasite, which treatment with 0.1 ml from pentostam (4 well as replications for each day).

3. Experimental groups: contains 0.2 ml culture of a parasite treated with four concentrations of 0.1 ml from NaCl NPs (2, 4, 6, and 8 µg/ml) with 4 replications for each concentration and each day.

The total number of parasites and the number of living parasites in each well were counted daily for four consecutive daysusing a hemocytometer and trypan blue stain (0.4%). Then, the Growth Index (GI) percentage was calculated as follows (17).

GI=(No. of treated promastigotes÷No. of non treated promastigotes)×100

GI: Growth index

2.4. Statistical Analysis

Using SPSS software did a statistical analysis, the results were analyzed with ANOVA analysis, LSD test, and Chi-square to determine the relationship among all experimental groups with a probability value less than 0.05%.

3. Results

The current study results revealed a significant effect of NaCl NPs concentrations on *L. major* promastigote during different 24, 48, 72, and 96hrs. It was found that the number of living parasites decreases with the increase of NaCl NPs concentrations. The highest concentration (8 µg/ml) gave a maximum inhibition reaching 45%, 69%, 78%, and 92%, respectively, with a significant impactat $P \leq 0.05$ compared with the control and pentostam groups. In contrast, thelowest 2 µg/ml concentration gave a minimum inhibition reaching 16%, 52%, 63%, and 73%, respectively. It wasalso compared with the control group (0.00% inhibition rate) and the pentostam group, with an inhibition index reaching 28%, 59%, 71%, and 86%, respectively (Figure 1 and Table 1). Using different levels of NaCl NPs reduced the growth of *L. major* promastigote, so with increasing time, its effectiveness increased. Using 6 and 8 µg/ml showed better performance in reducing growth than Pentostam.

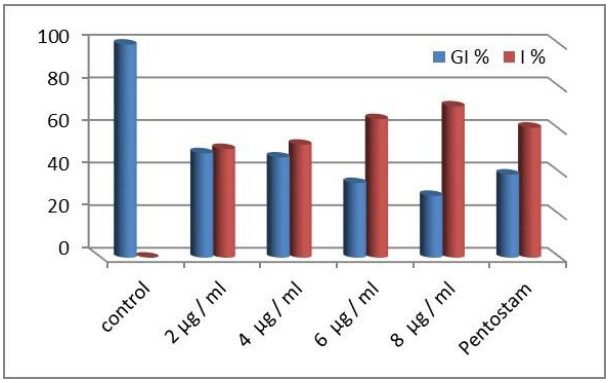

Figure 1. The rate of percentage of Growth index (GI%) and Inhibition index (I%) of *L. major* promastigote after treatment with different concentrations of NaCl NPs

Table 1. The effect of NaCl NPs concentrations on the growth of *L. major* promastigote, Growth index (G%), and Inhibition percentage (I%)

Groups		Hours				Rate ± SE
		24	48	72	96	
2 µg/ml	No. of promastigote	1.45×10^6	1.4×10^6	1.31×10^6	1.2×10^6	$1.34\times10^6 \pm 0.22\times10^6$
	G %	84	48	37	27	0.49± 0.05
	I %	16	52	63	73	0.51± 0.06
4 µg/ml	No. of promastigote	1.41×10^6	1.34×10^6	1.23×10^6	1.16×10^6	$1.29\times10^6 \pm 0.25\times10^6$
	G %	82	47	35	24	0.47±0.04
	I %	18	53	65	76	0.53±0.05
6 µg/ml	No. of promastigote	1.1×10^6	1.04×10^6	0.96×10^6	0.71×10^6	$0.95\times10^6 \pm 0.21\times10^6$
	G %	62	35	26	17	0.35±0.03
	I %	38	65	74	83	0.65±0.05
8 µg/ml	No. of promastigote	0.97×10^6	0.95×10^6	0.8×10^6	0.38×10^6	$0.78\times10^6 \pm 0.31\times10^6$
	G %	55	31	22	8	0.29±0.03
	I %	45	69	78	92	0.71±0.06
Pentostam	No. of promastigote	1.62×10^6	1.22×10^6	1.15×10^6	0.74×10^6	$0.09\times10^6 \pm 0.33\times10^6$
	G %	72	41	29	14	0.39±0.04
	I %	28	59	71	86	0.61±0.5
Control	No. of promastigote	1.9×10^6	3.0×10^6	3.8×10^6	5.0×10^6	$3.43\times10^6 \pm 1.33\times10^6$
	G %	100	100	100	100	100
	I %	0	0	0	0	0

4. Discussion

Today, many mineral nanoparticles have been prepared and researched. There are very few reports of common NaCl electrolytes. Assuming the dissolution of electrolyte nanoparticles in water and their more appropriate effect on physiological properties, the hypothesis of the effectiveness of this nanoparticle on *L. major* promastigote was proposed. The infection by Cutaneous Leishmaniasis had a wide range of diseases with obvious symptoms such as skin erosion, large ulcers, necrosis at lesion with opportunities for bacterial infection (6, 7). The treatment currently used against leishmaniasis depends on pentavalent Antimonial compounds like Pentostam. Many studies found that Pentostam has toxicity and several side effects, which continue until the recommended dose and treatment duration (18, 19). Therefore, it was necessary to look for other treatments or drugs that had a good effect against the parasite and had few side effects (20, 30).

The use of NaCl NPs concentrations on *L. major* promastigote at 8 µg/ml inhibited and reduced the number of *L. major* promastigotes. These results were consistentwith several similar studies that used other nanoparticles against *Leishmania* spp. *in vivo* (14, 16, 22, 25, 27). The outcomes of this studydemonstrate that the proliferation of the parasite was decreased with the increased concentrations of NaCl NPs during different incubation times of the parasite. In general, increasing concentrations of NaCl NPs with increasing incubation times leads to inhibition of *L.major* promastigote. This increase is due toNaCl NPspenetrating through the plasma membrane of parasites and causing biological effects on some metabolic pathways, like proteins and lipid synthesis and ion transport (2), and the effect on the mechanism of enzymes. The presence of NaCl NPs modified in cytoplasmic conditions leads to high stress inside cells, leading to cell membrane damage through the affecting on permeability and fluidity (1, 3). These changes destroyed cells and killed parasites, so the results showed a decrease in the number of *L. major* promastigotes during a long incubation time of 96 hours, more than 24 hours. The exact reasons can be said about the relationship between decreasing promastigotes growth and increasing concentrations in the experimental groups. The number of *L. major* promastigoteswasdecreased at high concentration

(8µg/ml) more than the low concentration (2µg/ml) compared with control and pentostam groups.

In conclusion, Sodium Chloride Nanoparticles (NaCl NPs) have a high inhibition against *L. major* promastigote at high concentrations (in vitro). The effect was 8µg/ml at 96 hours compared with the currently used drug against leishmaniasis (pentostam). The current study results give sparkling hope in treating leishmaniasis using nanoparticles as safe drugs.

Authors' Contribution

Study concept and design: I. A. M.
Acquisition of data: M. M. A.
Analysis and interpretation of data: I. A. M.
Drafting of the manuscript: I. A. M.
Critical revision of the manuscript for important intellectual content: M. M. A.
Statistical analysis: M. M. A.
Administrative, technical, and material support: M. M. A.

Ethics

All ethical procedures were approved by the ethics committee of the University of Al-Qadisiyah, Al-Qadisiyah, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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