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Evaluation of the immune response to silver nanoparticles against cutaneous leishmaniasis in albino mice Balbs/c

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Abstract---Leishmaniasis is an epidemic disease that affects the human body and is transmitted through the bite of the sandfly insect, is the most common and causes skin lesions mainly ulcers on the exposed parts of the body. The results of the examination in the level of immunoglobulins IgM and INF- γ concentration of one of the cellular kinetics showed that there was a significant increase of $P < 0.05$ in the sera of mice of the positive control group (first) infected with cutaneous leishmaniasis, compared with the negative control group (second). The results also showed that the drug pentostam (Sb), silver nanoparticle (AgNPs) and the loaded drug (AgNPs + Sb) had a significant effect on a decrease in the rate of immunoglobulins IgM and the lowest concentration rate was in the serum of mice treated with the drug loaded with silver nanoparticles. (AgNPs + Sb), while there was an increase in the average (INF - γ) concentration, where the highest concentration was in the group of mice treated with the drug loaded with silver nanoparticles (AgNPs + Sb) compared with the positive control group infected with cutaneous leishmaniasis, while at Compared with the negative control group, there was a significant increase in the rate of immunoglobulins and gamma interferon.

Keywords---immune response, silver nanoparticles, Leishmaniasis.

Introduction

Leishmaniasis is an epidemic disease that affects the human body and is transmitted through the bite of the sandfly insect and is in the form of three main types: Cutaneous Leishmaniasis, Mucocutaneous Leishmaniasis and Visceral Leishmaniasis (Karimi and Nabipour,2015; Alemayehu and Alemayehu,2017). Cutaneous Leishmaniasis, which is caused by two types of parasites, *Leishmania*

major and *Leishmania tropica*, is the most common and causes skin lesions mainly ulcers on the exposed parts of the body. In the Americas, the Mediterranean Basin, the Middle East and Central Asia (WHO, 2020). Nanobiotechnology using Nano-sized particles has been developed to treat many diseases including human tumors. Nanoparticles are very useful due to their structure and size as well as unique biological effects (Ravichandran *et al.*, 2016). Silver nanoparticles (AgNPs) have achieved special importance in scientific research (Ahmed *et al.*, 2016). Studies have shown the direct and harmful effects of silver nanoparticles on the different forms (amastigotes, promastigotes) of the parasite *Leishmania Tropica*, and the parasites' mortality increases with increasing the concentration of silver nanocomposites used *ex vivo* (Gharby *et al.*, 2017).

Materials and Methods

RPMI-1640 medium obtained ready-made from ABI (Armerica) company, in which 10% each of Fetal Bovine Serum, antibiotics penicillin and streptomycin were added, was used to develop the promastigotes phase of *Leishmania* parasite. Silver nanoparticles were prepared by dissolving 0.0285 gm of (Sodium borohydride) in 10 ml of deionized water in an ice bath, then 0.4 mg of (polyvinyl pyrrolidone) is added as a stabilizer for the reaction, then 0.0214 g of (silver nitrate) is dissolved in 10 ml of distilled water and then mixed, then the final solution is placed on a magnetic stirrer and stirred at 1500 rpm for one hour at 50-60°C until the resulting solution turned blackish-brown in color and then dried in an electric oven (Zhu *et al.*, 2011). Several methods were used to measure nanoparticles, including X-ray diffraction, Scanning Election Microscope, transform infrared Microscope and Fourier Atomic Force.

It was used in the experiment of white male mice that were divided into 11 groups, each group contains 8 mice, and the concentrations ng/ml 100,200,300 pentostam and silver nanoparticles (AgNPs) and loader were prepared as follows: The first group (positive control) was injected each 1 mL animal subcutaneously from anterior flagella and left untreated, group II (negative control), group III pentostam (Sb) at 100 µg / ml, group IV (AgNPs) at 100 µg / ml, and group V (Sb + AgNPs) . 100 µg / ml, group VII pentostam (Sb) at a concentration of 200 µg / ml, group VIII (AgNPs) at a concentration of 200 µg / ml , group VIII (Sb + AgNPs) at a concentration of 200 µg / ml, group IX pentostam (Sb) at a concentration of 300 µg / ml, group The tenth group (AgNPs) at a concentration of 300 µg/ml, the eleventh group (Sb+AgNP5) at a concentration of 300 µg / ml, the immunoassays for both IgM and IFN- γ were measured using (ELISA) technique.

Results and Discussion

In the average, the results shown in Tables (1, 2) showed that there was a significant increase of <0.05 P IgM and IFN- γ in the positive control group (first), which amounted to (9.08 ± 193.5 mg/dl), (2.83 ± 69.39 ng/dl). Respectively, when compared with the negative control group (second), which reached (2.38 ± 68.3 mg/dl), (8.63± 42.01 ng/dl), respectively, as many researchers indicated in the course of their study on the effect of leishmaniasis infection on the humoral immune response. These results are consistent with the results of the study that

showed that the levels of immunoglobulins in the blood serum (IgG and IgM) were elevated in patients suffering from both types (*L. Major*, *L. Tropic*) of cutaneous leishmaniasis (Al-Qadhi *et al.*, 2015).

Also showed at Soong (2019) and Kima and Boussoffara *et al.* (2013) an increase in INF- γ concentration in leishmaniasis. The study also indicates that the response of Th2 cells is in favor of the parasite and helps to stabilize it in the host, while the response of Th1 cells is lethal to the parasite larvae. However, it is not as simple as it seems, as there is a mixed Th2/Th1 response in the infected host (Novais *et al.*, 2022 ; Costa-da-Silva *et al.*, 2022 ; Tafaghodia *et al.*, 2016), the study confirmed the presence of two different cell types in T helper cells of mice, type I (Th1) cells produce the cytokines IL-2, IFN-TNF- β , while helper cells type II (Th2) secrete cytokines IL-10. IL-5 IL-6 IL-4 (Carneio *et al.*, 2016) .

The results of the current study showed in Tables (1) that the eleventh group silver nanoparticles (Sb + AgNPs) had a significant effect on a decrease in the rate of IgM, as it was the lowest rate in the sera of mice if it reached (2.79 ± 68.32 mg/dl) on the respectively when compared with the positive control group (first) (9.08 ± 198.5 mg/dl), and these results are consistent with the results of both researchers, (Silva-Barrios *et al.* , 2016; Taher, 2006) when using treatment in low mean value of IgM globulin and IgG whose values were higher than those of the control group, and that the activity that the interaction between nanoparticles and the immune system leads to many useful medical applications such as vaccines, drug or antigen delivery, or treatment of infections and autoimmune diseases (Dobrovolskaia *et al.*, 2016). This was confirmed by several studies, (Abulaihaiti *et al.*, 2015), (Himly *et al.*, 2017) including They concluded from experiments with a group of free and loaded nanocomposites that stimulate immune responses over time, that Th2 lymphocytes, which secrete cellular dynamics that affected the growth and differentiation of B cells) Which is considered to have a significant effect on the release of large quantities of antibodies, while the results of the study presented in Table (2) noted that the drug Pentostam, free silver nanoparticles, and the free drug loaded with them had a significant effect in increasing the rate of interferon gamma concentration (INF- γ), where The highest concentration was in the group of mice treated with the drug loaded with silver nanoparticles (Sb + AgNPs), any group (11) which reached (2.01 ± 125.06 ng/dl) compared with the positive control group, which reached (2.83 ± 69.39 ng/dl). There was a significant increase in the concentration rate of interferon gamma when compared with the negative control group, which reached (8.63 ± 42.01 ng/dl).

The activity performed by the interaction between nanoparticles and the immune system leads to many beneficial medical applications such as vaccines, drug or antigen delivery, or treatment of infections and autoimmune diseases (Dobrovolskaia *et al.*, 2016). INF - γ and a decrease in IL-10 production when studying the effect of *Fusarium graminearum* loaded on silver nanoparticles when compared with pentostam in mice infected with visceral leishmaniasis. Infected mice unresponsive to treatment (Mohammed *et al.*, 2019), while Liu *et al.*, (2009) demonstrated through his study on the effect of polyhydroxy-metallophyleneol nanocomposites in mice that led to an immune response to Th1 cells by increasing the production of synthesis Cytokinetics of IFN- γ , 2-TNF- α , IL-

2 and conversely in decreased production of IL-4, IL-5, IL-6, due to decreased response of Th2 cells.

Table (1) The activity of pentostam and free silver nanoparticles loaded with pentostam at different concentrations IgM in sera of male mice infected with cutaneous leishmaniasis

groups	Arithmetic mean \pm standard deviation (study groups)	The first group is positive control	t-test Value	Probability Value
2	2.38 \pm 68.3		29.82	0.000
3	3.31 \pm 118.9		7.71	0.001
4	3.02 \pm 132.84		6.33	0.002
5	2.62 \pm 92.82		10.65	0.001
6	3.61 \pm 107.78	91.08 \pm 193.5	8.77	0.003
7	8.81 \pm 97.48		7.58	0.001
8	3.33 \pm 71.16		12.64	0.001
9	3.85 \pm 94.98		9.98	0.002
10	3.62 \pm 103.44		9.21	0.001
11	2.79 \pm 68.32		13.17	0.004

Table (2) The activity of pentostam and free silver nanoparticles loaded with pentostam at different concentrations of INF- γ in sera of male mice infected with cutaneous leishmaniasis

groups	Arithmetic mean \pm standard deviation (study groups)	The first group is positive control	t-test Value	Probability Value
2	8.63 \pm 42.01		25.22	0.000
3	2.83 \pm 68.39		0.11	0.930
4	4.29 \pm 66.54		0.55	0.598
5	2.37 \pm 104.56		9.50	0.001
6	1.41 \pm 77.95	2.83 \pm 69.39	2.70	0.036
7	2.94 \pm 73.22		0.93	0.376
8	2.37 \pm 108.67		10.61	0.001
9	1.80 \pm 90.75		6.35	0.002
10	2.15 \pm 86.69		4.85	0.001
11	2.01 \pm 125.06		16.02	0.001

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