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Evolution of gold nanoparticles as a drug against cutaneous leishmaniasis *in vitro*

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Abstract

This study is conducted between 13/12/2021 to 5/5/2022 in the city of Samarra, and 83 blood samples are collected for people with leishmaniasis. Serological tests are carried out and it is found that 69.87% of those infected had specific antibodies IgM and 30.13% had IgG antibodies. The results of the study show the inhibitory efficacy of gold nanoparticles at size 0.15 on average growth of 1.56× 106 cells/ml in time 96, which inhibited the growth of the parasite since the start of the experiment at the probability level of p≤ 0.05 While the volume of 0.20 µL show a high inhibitory effect on the growth of the Promastigote of the leishmania parasite at an average growth of 0.85×106 cells / ml in time 72 from the start of the experiment at a significant level p≤ 0.05. The highest level of inhibitory effectiveness of the growth of the Promastigote of the f of the leishmania parasite is at the volume of 0.30 µL, where the average growth become 0.61 cells / ml. in the 48th time at the start of the experiment and this volume is the best size to affect the growth of the parasite in the agricultural medium compare to the effect of the drug Betamethasone, which inhibited the growth of the parasite at the concentration of 0.50 µL to 0.24×106 cells / ml at the 48th time from the start of the experiment at a significant level p≤ 0.05.

Introduction

Leishmaniasis is prevalent in low-income countries and is one of seventeen important tropical diseases classified by the World Health Organization. The disease is endemic in 98 countries, but about 75% of cases of leishmania in humans have been recorded in 10 countries, namely Afghanistan. Algeria, Brazil, Colombia, Costa Rica, Ethiopia, Iran, Iraq, Peru, and Syria [1].

Leishmania has a lot of health problems and despite their seriousness and speed of spread, it remains neglected globally [2]. Leishmania parasites spread and continue through a complex life cycle between the Female sand fly Phlebotemuos ssp. and its vertebral havens, caused by A single-cell parasite protozoan, are compulsory to intrude into living cells and are shared with humans and animals zoonotic and transmitted from one host to another by biting a female sandfly [3].

Leishmaniasis has three clinical forms: cutaneous leishmaniasis, mucocutaneous leishmaniasis, and visceral leishmaniosis [4]. It usually affects exposed areas of the skin that

develop into pink papules and then nodules or plaques, and eventually form an ulcer with wide boundaries [5]

When the parasite promastigote stage penetrates the body stimulates both the cellular immune and humoral immunity of the host and is swallowed by macrophages .The process of phagocytosis devouring takes 4-8 hours where of the parasite adheres to the surface of the phagocyte cell and then becomes surrounded and devoured by pseudopodia and thus is surrounded by a phagosome gap and turns into an amastigote stage [6] After that the Amastigote phase begins to fission until the phagocytes are filled with the parasite and then explode and release many Amastigote phases with many types of peroxides and free radicals as remnants of the parasite to be swallowed by other phagocytes and so the process continues until the onset of pathological symptoms and the confined period between the entry of the parasite until the onset of pathological symptoms is called the Incubation period [7] . It has been shown that phagosome state gaps containing leishmaniasis parasites fuse with lysosome state particles and this process do not appear to affect the parasite that continues to multiply within particles phagolysosome gaps resulting from this fusion [8].

The immune response is shown by cells inside cells mainly within macrophages and the outcome of infection depends on whether the host responds primarily in response to Th2 and Th1. Helper, as the immune response to skin leishmaniasis, is complex, although it accelerates recovery, some responses worsen the disease, and the immune response of the host depends on several factors, including the genetic difference of the host and the genetic difference between the strains and types of the parasite itself, and other factors including the location and size of the injury and the number of infectious stings [9].

The World Health Organization has recommended treatment with Pentavalent antimonial compounds, including the drug pentostam Betamethasone and Glucantime-commonly used for people with cutaneous leishmaniasis and uses 20 mg of manna per kg for 10-14 days and the treatment can be given intravenously or intramuscularly and these treatments are characterized by side effects such as elevated toxicity and loss of effectiveness with the increase in the duration of treatment, also used to treat leishmaniasis some antibiotics Amphotericin B and Pentamidine and also It has negative effects such as Betamethasone [10] .

Many topical treatments are also used to treat skin ulcers and the use of ointment, which consists of Promomycin, Methylbenzathonium 12%. chloride and paraffin soft white oil, has eliminated 75% of the parasites inside the ulcer and this treatment is commercially called Leshcutan [11].

The persistence of the disease and its spread and the effects it leaves on the victim of physical impact and psychological factors led to urging researchers to find other alternatives to chemotherapy Where innovations in the disciplines of medicine and science have made a scientific shift (the use of medical applications of nanotechnology for human well-being the field of treatment used what is known as nanomedicine, that is, the employment of nanoparticles loaded with drugs such as metal nanoparticles, liposomes, nanoparticles, nanoparticles. Polymeric nanoparticles efficiently deliver treatment to target sites for the treatment of diseases including leishmaniasis [12]. Zinc oxide particles have been used in the treatment of leishmaniasis and have proven effective [13]. As well as treatment with silver

nanoparticles against the leishmaniasis parasite [14], as noted [12]. The use of silver oxide nanoparticles has promoted healing and removal of parasites in mice infected with cutaneous leishmaniasis.

Gold is one of the rarest minerals on Earth. Moreover, it is considered to be one of the safest and Is characterized as chemically inert [15]. Gold nanoparticles are one of the most commonly used and studied nanoparticles where gold metal is one of the most stable minerals making it the most used in scientific research and study [16]. The production and synthesis of gold nanoparticles are subject to a base and are from the top down in this process. Gold salts are reduced by the presence of stabilizing agents, which play a role in preventing the agglomeration of gold particles with each other [17].

Aim of the study

The current study aimed to see the impact of gold nanoparticles on leishmaniasis.

Material and Methods

At the time of collecting 83 blood samples of people with dermatology and conducting serological tests.

The -free parasite Amastigote was diagnosed in the direct smear taken from the Lesion and dyed Giemsa stain as pictured (1).

Methods of work

The culture media was used in the study

When the parasite was cultured in the implant medium, the results showed the growth of the parasite in the concentrated cultivated medium (RPMI -NNN) better than the rest of the media used in this study, the samples were examined after 96 hours of X40 strength and obtained a good growth rate for the parasite because the medium is rich in the essential nutrients needed by the parasite.

Parasites Number Calculation

1) 1 ml of the planting medium, which contains the leishmaniasis parasite, was taken to a second tube, and 9 ml of distilled water was added to it into the tube and shaken well to become diluted in a ratio of 1:10.
2) 10 µL was taken using a pipette from the previous tube, placed on the calculation box for Hemocytometer blood cells and then placed the in glass cap box to calculate the number of parasites under a microscope at a magnification force of 40X and according to the following equation [18].

Gold nanoparticles

The 60nm gold Nano solution with a concentration of 3.127 manufactured by the American company (Sigma Alorich) was used as a therapeutic attempt against the leishmaniasis parasite in the vitro The Several different sizes (0.05, 0.1, 0.1, 0.15, 0.2 and 0.3) were taken from the gold Nano solution and was added to the different culture media used in the study containing the leishmaniasis parasite in the anterior phase of the Amastigote. The growth of the parasite was followed for 5 consecutive days and the results were compared with control samples for the growth of the parasite.

Results

Immunological test results

This study showed that 69.87% of people with IgM and 30.13% had IgG antibodies as in table 1, and this result was consistent with what I found (11). The direct examination of the lesion swab using microscopy is an excellent standard diagnostic method, and also agreed with [17] in the presence of the parasite within the phagocytes infected with the circular or oval shape upon direct examination.

Table1: Percentage of immunoglobulin in cutaneous Leishmania

Percentage	Number	Antibodies
%30.13	25	IgG
%69.87	58	IgM

There are significant differences at the level of P<0.05

Results of the growth rate of the parasite in the axial cultivated medium (NNN – RPMI)

The results in the cultivated medium showed that the growth rate of the leishmaniasis parasite as in Table 2 gave a highly significant difference at the level of p≤ 0.05compared to the media in which the parasite was developed, it showed the growth rate in the 24-hour (2.6 × 106 cells/ml) while in 48 hours (3.9 × 106 cells/ml), at time 72 hours (5.2 ×106 cells/ml), in 96 hours (3.1 ×106 cells/ml) and at time 120 hours (1.9 ×106 cells/ml).

Table 2: The growth rate of the parasite in the Media used at different times (hours)

Average media	Time / hours					Culture media
	120	96	72	48	24	
106 *1.7 C	106 *0.98 g	106 *1.7 ef	106 *2.6 d	106 *1.9 e	106 *1.2 g	RPMI
106 *2.3 B	106 *1.4 fg	106 *2.1 e	106 * 3.4 bc	106 *2.6 d	106 *1.8 e	NNN
106 *3.3 A	106 *1.9 e	106 *3.1 c	106 *5.2 a	106 *3.9 b	106 *2.6 d	RPMI – NNN
	106 *1.4 d	106 *2.3 c	106 * 3.7 a	106 *2.8 b	106 *1.9 c	Average time

The reason for this remarkable rise in the growth rate is due to the fact that two basic mediums of growth of the parasite have been combined, each of which is complementary to the other in terms of the presence of essential nutrients, as well as the lack of addition of antibiotics may have a role in not weakening the parasite and affecting its virulence and activity, adding maltose to the medium may have an effect in increasing the activity and effectiveness of the parasite (5).

Impact of gold nanoparticles on the activity and vitality of the parasite in Promastigote of the Leishmania parasite in the culture media (NNN-RPMI 1640). This study used different volumes of gold nanoparticle solution with a concentration of 3.127 with a size of 60nm. the Promastigote was treated to the leishmaniasis parasite at each of the following sizes (0.05, 0.10, 0.15, 0.20, 0.30) microliter and follow-up growth over five days, shown by the results and as in Table 3.

Table 3: Sizes of gold nanoparticles used to inhibit parasite growth in the NNN-RPMI 1640)

Average concentration MD	120	96	72	48	Time 24	Concentration
106×3.16 a	106×1.9 C	106×2.9 c	106×5 b	106× 3.4 a	106× 2.6 a	0.05
106×2.92 b	106×1.7 E	106×2.4 e	106×4.8 b	106×3.1 a	106× 2.6 a	0.10
106×1.56 c	106×0.64 C	106×0.91 c	106×1.6 b	106× 2.1 a	106×2.6 a	0.15
106×0.85 d	0 D	0 d	106×0.56 **c**	106×1.1 b	106×2.6 a	0.20
106×0.61 e	0 C	0 **c**	0 c	106×0.48 b	106×2.6 a	0.30

The size of 0.05 and 0.10 micro liters did not give any moral difference as an inhibitory effect of phase growth in Promastigote leishmania parasite at p≤ 0.05. The results of the study showed an inhibitory efficacy of gold nanoparticles at size 0.15 on the average growth of 1.56 × 106 cells/ml in time 96 which inhibited parasite growth from the start of the experiment at p≤ 0.05probability level.

The size 0.20 microliter showed a high inhibitory effectiveness on phase growth in the Promastigote leishmania parasite at average growth 0.85 × 106 cell/mL in time 72 from the start of the experiment at p≤ 0.05morale level. The highest level of inhibitory effect of the growth of the Promastigote leishmaniasis parasite was at the volume of 0.30 µL, where the average growth became 0.61 cells / ml in the 48th time of the start of the experiment and this volume is the best size to affect the growth of the parasite in the agriculture media compared to the effect of the drug pentosam, which inhibited the growth of the parasite at the concentration of 0.50 µL to 0.24×106 cells/ml at the 48th time from the start of the experiment at a significant level p≤ 0.05.

Discussion

Nanoparticles have been used as an alternative approach to supplementing or replacing special malarial antibiotics in the treatment of infectious diseases. Studies on the impact of nanoparticles of various types have shown an important role in the fight against leismania. These substances can improve the diagnosis of leismania by increasing specific sensitivity in molecular and immunological diagnostic tests during treatment [19].

The results of this study are consistent with his findings [17]. in the use of gold particles silver and metal oxide in inhibiting the growth of leishmania plasmodium parasite and insect larvae as agreed with the results [8]. in the effect of gold nanoparticles paired with chrysin on inhibiting the growth of leishmania parasite as an effective treatment against parasite and its low damage on mammal macrophages. These results are agreed with an experimental study to evaluate the effectiveness of gold nanoparticles against dermal champagne. The results show a moral decline in numbers of the Promastigote parasite compared to the control group and resulted in a decrease in mortality among infected mice [17].

The reason for the decrease in parasite numbers and inhibition of its growth is likely due to the toxic effect of gold nanoparticles. Gold and silver nanoparticles have proven promising results in the treatment of many different types of parasitic infections and have shown great effectiveness in this aspect by using different methods to eliminate parasitic membranes, including damage to the parasitic membrane. Nanoparticles can cause endoplasmic network strain resulting in multiple cell dysfunction that causes disturbances in their functions, including protein accumulation and apoptosis (8). Nanoparticles can enter the inside of cells by phagocytosis and over time dissolve these intracellular nanomaterials triggering the release of transitional metal ions capable of generating ROS. The entry of nanoparticles into Lysosomal may also destabilize and release to cellular acidification degradation and cellular acidification enzymes and may cause nanoparticle-based cellular acidification rupture to release cathepsin protein that stimulates silence or autophagy [6]. The association of nanoparticles with proteins can impair protein functions by changing its structure or structure. This change in protein structure results in the exposure of amino acid regions or sites that are usually hidden within the folded protein (13).

Research previously studied on nanoparticles and mineral compounds for the treatment of leshmaniasis has shown a fundamental view of the fact that these compounds activate Trypanothione metabolic enzymes that are important for leshmaniasis survival (20). Tryponothione reductase (TR) is one of the best intended targets for finding new drugs against leshmaniasis. This enzyme is essential for the survival of the parasite in the human host and is a molecule used by the peroxidase system to neutralize hydrogen peroxide produced by pharyngeal cells during infect (9). Gold nanoparticles have been widely used as anti-parasites and have shown high toxicity efficacy against leishmania, Malaria, Toxoplasma, Trypanosomiasis and Cryptosporidium (16). The effect of particle activity for gold, silver and platinum was also studied as an anti-trypanosomal activity, and showed high effectiveness in the particles being 200 times higher selective activity against parasite versus mammal cells (21). Use of gold nanoparticles as a therapeutic attempt in experimental animals. The results showed a moral decrease in the number of parasites in stool and small intestines of mice infected with giardia and a marked improvement in the infected intestinal mucosa (1). The gold nanoparticles did not reveal any cellular toxicity in the phagocytes of the mice but exercised significant anti-parasitic activity in the front-stage whip and whip-free phase with morphological deformation of parasites and fewer sores appearing on affected mice after processing (11).

Conclusion

The study has proved that there is an effect of gold nanoparticles on the parasite's growth in vitro at a size of 0.20 and 0.30 microliters. Gold nanoparticles also have a parasite growth inhibitor effect. Gold nanoparticles can be used with some treatments in many pathological situations and have an effective and strong effect against microbial biota in general and parasites in particular. The researcher recommends studying the impact of gold nanoparticles on the parasite in the organism and knowing the impact of dermal lashmania on certain elements such as Bromine and vanadium.

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تقييم جزيئات الذهب النانوية كدواء ضد داء الليشمانيا الجلدي في المختبر

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الخلاصة:
بدأت هذه الدراسة من تاريخ 2020/12/13 ولغاية 2021/5/5 تم جمع 83 عينة لأشخاص مصابين باللشمانيا الجلدية مراجعين لمستشفى سامراء العام، أجريت الاختبارات المصلية ووجد أن 69.87٪ من المصابين لديهم أجسام مضادة محددة IgM و30.13٪ لديهم أجسام مضادة IgG. واظهرت بعضها نتائج ايجابية وتم استزراع الطفيلي في اوساط زرعية مختلفة ومتابعة النمو لدة 5 ايام متتالية وجد من خلال الدراسة ان الوسط الزرعي المحور RPMI 1640 +NNN هو افضل وسط لنمو الطفيلي من بقية الاوساط حيل بلغ متوسط معدل النمو الى (5.2×106 خلية / مل) في الزمن 72 اما في الوسط RPMI 1640 كان متوسط معدل النمو(2.6×106 خلية/ مل)عند الزمن 72, وفي الوسط NNN بلغ (3.4×106 خلية/ مل) عند الزمن 72 .تم دراسة تأثير جسيمات الذهب النانوية ذات التركيز 3.127 nm على الطفيلي في الوسط الزرعي المحور RPMI 1640 +NNN باستخدام تراكيز مختلفة (0.05 ,0.10, 0.15, 0.20, 0.30) مايكروليتر وجد ان التركيز 0.20 مايكروليتر كان له تأثير مثبط لنمو الطفيلي في الزجاج عند الزمن 72 من بدء التجربة اما التركيز 0.30 ما يكرو ليتر قد ثبط نمو الطفيلي عند الزمن 48 من بدء التجربة .