# Nanoparticles Used in Treatment and Diagnosis of Leishmaniasis in Iraq: A Review

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# الدقائق النانوية المستخدمة في تشخيص و علاج داء الليشمانيات في العراق: دراسة مرجعية

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## Abstract

The aim of this paper is to identify Nano-particles that have used in diagnosis and treatment of leishmaniasis in Iraq. All experiments conducted in this field were based on the following nanoparticles: gold nano-particles, silver nano-particles zinc nano-particles and sodium chloride nano-particles. Most of these experiments were reviewed in terms of differences in the concentrations of nano-particles and the method that was used in the experiments whether it was *in vivo or in vitro*. These particles used in most experiments succeeded in inhibiting the growth of *Leishmania* parasites.

**Keywords: Diagnosis**, *Leishmania*, **Nano-particles, Treatment**

## المستخلص

هدف البحث الاتي الى التعرف على الجزيئات النانوية المستخدمة في تشخيص وعلاج داء الليشمانيات في العراق. اعتمدت جميع التجارب التي أجريت في هذا المجال على الدقائق النانوية التالية: جزيئات الذهب النانوية، وجزيئات الفضة النانوية، وجزيئات الزنك النانوية، وجزيئات كلوريد الصوديوم النانوية. تمت مراجعة معظم هذه التجارب من حيث الاختلافات في تراكيز الجزيئات النانوية والطريقة التي استخدمت في التجارب سواء كانت داخل الحي اوخارج الحي. وقد نجحت هذه الجسيمات المستخدمة في معظم التجارب في تثبيط نمو طفيليات الليشمانيا.

**الكلمات المفتاحية: تشخيص, طفيلي اللشمانيا, دقائق نانوية, علاج**

## Introduction

Endoparasites and ectoparasites are two types of parasites that pose a threat to human health. Parasites can reside within their host or on its surface. Approximately 200,000 individuals die as a result of parasite illnesses, which affect 3.5 billion people annually. Significant illnesses like trypanosomiasis, leishmaniasis, malaria, and Toxoplasmosis—all of which have high rates of illness and death in developing countries—are caused by these parasites. Ectoparasites are arthropods that either transmit certain parasites or cause diseases while endoparasites are organisms that live in biological tissues and cause serious health problems (Matthee and Kransov, 2009; Ikejiofor, *et al*., 2021). It is widely recognized that human behavior, population shifts, and environmental changes significantly affect the spread, dispersal, occurrence, and incidence of parasitic diseases. Most parasitic diseases and their modes of transmission have also been discovered (Thompson, *et al*., 2005). Numerous treatments for parasitic infections have been attempted; however, because of parasite resistance and a range of adverse drug reactions, most treatments are no longer effective. Chemotherapeutic drugs and ethnobotanicals were two previous treatments for parasites. Resistance to chemotherapy treatments has evolved in parasites. Thus, the ideal strategy for addressing the previously described issue of parasitic illness is the development of novel biotechnologies to enhance the specificity, efficacy, and tolerability of diagnostic tests as well as the safety of existing anti-parasitic drugs (Fidock, *et al*., 2009; Huijben, *et al*., 2010). "Nano-technological sciences" (or "nanoparticle-based science") is the phrase used to describe the technological application of physical, chemical, or biological systems at sizes varying starting from submicron

diameters to individual atoms or molecules. Comprises studies on drug administration and medical diagnostics (Surendiran, *et al*., 2009; Qasim, *et al*., 2014; Yezdani, *et al*., 2018). Nanotechnology-based pharmaceutical dosage forms are now being created and used widely worldwide. The field of nanotechnology encompasses a wide variety of various areas of competency, including the application of innovative technologies in medicine, engineering, and veterinary medicine. New nano-technological products, such as vaccines, recombinant proteins, and other medicinal alternatives, enable a safer environment for humans and/or animals. Nanotechnology has significantly advanced the growth of several industries, including biological applications such as tissue engineering (Kingsley, *et al*., 2013), medication transport, bio-imaging, and nano diagnostics (Dar, *et al*., 2021; Mirjalili, *et al*., 2012; Nawala, 2014). Applications of nano-diagnostics for infectious disorders are gaining increasing interest due to its unique features in high sensitivity and early detection. Because of the current threat to public health posed by antibiotic resistance, numerous fields have been established along with significant advancements in the quest for effective and novel treatments (Rossolini, *et al*., 2014; Aslam, *et al*., 2018). Nanomedicines are an important tool in the development of antibacterial medications, with many advantages (Wang, *et al*., 2015). Many parasites are spread in Iraq, whether they are protozoa or helminthes (Al Saqur, *et al*., 2016; Al Saqur, *et al*., 2017; Al Saqur, *et al*., 2020; Al-Warid, *et al*., 2022). Many researches related to diagnosis and treatments have been done (Al shakir and Zghair, 2015; Bedair and Ali, 2020; Abdullah and Alqaisi, 2022). One of the most important of these parasites is the *Leishmania* parasite. *Leishmania* is an obligatory protozoan parasite causes leishmaniasis (Steverding, 2017). Many different parasite species belong to

this genus have been recognized. Twenty one of them are considered as pathogenic agents (Colmenares, *et al*., 2002). *Leishmania donovani, Leishmania amazonensis, Leishmania aethiopica*, and other species are some of those that cause leishmaniasis. *Leishmania* has two primary stages: amastigot and promastigot. The promastigte is elongated and flagellated, whereas the amastigote is spherical, small, and non-motile. The transmission of *Leishmania* occurred once a sand fly feeds on the blood of infected subjects whether animal or human (Gossage, *et al*., 2003). The metamorphosis happens when the parasite moves from the amastigote stage to the promastigote, which takes 5 to 26 days (Ambit, *et al*., 2011). The illness results in ulcers as well as the infection may affect other body region (van Griensven, *et al*., 2019). Mucosal leishmaniasis (ML), cutaneous leishmaniasis (CL), and visceral leishmaniasis (VL) are the three main types of leishmaniasis. The signs of ML take long time to be noticed, characteristically 1 to 5 years. (Remadi, *et al*., 2017). In the most frequent variety, VL symptoms, which include weakness, weight loss, fever, hepatosplenomegaly, lesions, and swollen lymph nodes, develop in around 2 to 6 months (Kobets, *et al*., 2012) and in CL, the symptoms noticed a few weeks after the individual is bitten by the vector. In endemic areas of the world, 0.2–0.4 and 0.7–1.2 million cases of VL and CL, respectively, have been documented. Several nations, including Algeria, Afghanistan, Colombia, Syria, Brazil, Iran, Ethiopia, Costa Rica, North Sudan, and Peru, have reported about 75% of the estimated worldwide prevalence of CL, whereas Bangladesh, India, South Sudan, Ethiopia, and Brazil have reported more than 90% of the cases of VL (Piggot, *et al*., 2014)]. In Iraq, both the cutaneous leishmaniasis (Baghdad boil) and visceral leishmaniasis (Kala-azar) are common. The eastern provinces of Iraq: Diyala,

Wasit, Missan, and Basrah were reported to have high prevalence rates of leishmaniasis (Al-Warid, *et al*., 2011; Al-Warid, *et al*., 2017). Except for three northeastern provinces, all of Iraq is affected by cutaneous leishmaniasis (CL), which is spread by the sand-fly species *Phlebotomus papatasi* and *P*. *sergenti* (Abd, et al., 2020). Both zoonotic CL caused by *L. major* and anthroponotic CL caused by *L. tropica* were documented in Iraq (Shahatha and Saleh, 2018). There were 955 and 625 cases of CL, respectively, in 2000 and 2001, down from 8779 cases in the peak year of 1992. Zoonotic cutaneous leishmaniasis (ZCL) is more common and mostly affects rural areas, especially in the northern and southern provinces of Iraq (Al-Obaidi, *et al*., 2016). Cases of anthroponotic cutaneous leishmaniasis (ACL) predominately arise in the suburbs of big towns. A significant CL outbreak occurred in 2008, with 300 cases being recorded from the region of Qadisiyah (Al-Obaidi, *et al*, 2016). This review will address some of the nanoparticles utilized in the diagnosis and treatment of leishmaniasis in Iraq due to the wide range of uses of nanotechnology, particularly in diseases such as parasites.

## Gold nanoparticles

Gold nanoparticles (AuNPs) are very minute gold particles with a diameter ranged between 1 to 100 nm. Au NPs have been used to target delivery of chemotherapeutic agents, complement radiation and thermal therapy, and improve contrast for *in vivo* imaging of the tumors. Among the rarest minerals in the world is gold. It's also regarded as one of the safest and is described as chemically inert (Sardar, *et al*, 2009). Since gold metal is one of the most stable materials, it is most frequently utilized in scientific research and study, making gold nanoparticles one of the most widely used and

studied nanoparticles (Moreira, *et al*., 2014). The synthesis and manufacture of gold nanoparticles follow a top-down method and are dependent on a base. Gold salts are reduced by the presence of stabilizing agents, which play a role in preventing the agglomeration of gold particles with each other [Huang and Yang, 2004). In an attempt to treat the *Leishmania* parasite *in vitro*, Abdul Aziz, *et al*., (2022) used a 60 nm gold nano solution with a concentration of 3.127. From the gold nano solution, numerous sizes (0.05, 0.1, 0.1, 0.15, 0.2, and 0.3) were taken-out and presented to the various culture media that were used in the study. The parasite's growth was monitored, and the results were compared with those of control samples. Their results confirmed that gold nanoparticles with a size of 0.15 were active in inhibiting the growth of *Leishmania* parasites, which had been rising at an average rate of 1.56 × 106 cells/ml in time 96 hours. At time 72 hours from the beginning of the trial, the volume of 0.20 µL had a strong inhibitory influence on the Promastigote of the parasite, with an average growth rate of 0.85×106 cells / ml. The highest level of inhibitory efficiency of the growth of the Promastigote of the f of the *Leishmania* parasite is shown 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 trial and this volume is the best size to affect the growth of the parasite in the cultivated medium compare to the effect of the Betamethasone, which inhibited the growth of *Leishmania* 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. On the other hand, some investigators examined gold nanoparticles (Au NPS) as a probe to detect *Leishmania* parasites in canine blood samples (Al-Ardi, 2022). In his work, 4 nmol of thiolated oligonucleotides and 1 ml of AuNPs aqueous

solution were used to create AuNPs probes, which were subsequently bound by the sequence of oligonucleotide probes. Three replicates of 10 µl of 23 ng / µl of *Leishmania* spp. were utilized by him. Based on his findings, *Leishmania donovani* was positive in 10 out of 10 samples, with a 100% sensitivity and specificity. While 8 out of 10 cutaneous *Leishmania* samples were positive, with 80% specificity and sensitivity.

## Silver nanoparticles

Silver nanoparticles are silver particles with a size ranged between 1 to 100 nm. Depending on the intended use, nanoparticles can take on a range of forms. Spherical silver nanoparticles are often used; thin sheets, diamond, and octagonal shapes are also regularly encountered. AgNPs are mostly used as bio-sensors, vaccine adjuvants, anti-diabetic mediators, and in the elevation of wound and bone healing in addition to antibacterial and anticancer therapy (Das, *et al*., 2020). Al-Saeedi and Saheb (2017) conducted a study to investigate the cytotoxicity of macrophages in response to exposure to Ag NPs and *L. tropica*. Following the exposure of macrophages to *L. tropica*, concentrations of Ag NPs (8, 4, 2, 1, 0.5, and 0.25 µg/ml) were utilized in a serially diluted manner. The vitality of macrophages and *Leishmania* (promastigote and amastigote) was confirmed using the Thiozolyl Blue Tetrazolium bromide (MTT) test. Compared to the control group, the viability percentage of macrophages has raised up by 104.53 ± 4.62%. Their findings showed that Ag NPs were usefully active in endorsing the growth of promastigote and amastigote types. Ag NPs' 50% inhibitory concentration (IC50) on promastigotes was determined to be 2.988 µg/ml, and their IC50 on amastigotes after *in vitro* macrophage infection was assessed to be

2.584 µg/ml. According to their investigation, Ag NPs stimulated macrophages in a way that withdrawn the growth of *L. tropica in vitro* after parasite infection. While Mohammed (2017) studied the effectiveness of AgNPs isolated from *Sphingomonas paucimobilis* against *L.donovani* both *in vivo* and *in vitro*. She compared pentostam with AgNPs' efficiency. According to her results, nanoparticles significantly lower the concentration's promasitgote percentage (0.88 mg/ml). The amount of *L.donovani* in the spleen was noticeably lower with pentostam and silver nanoparticles than with the control. In contrast, Hannon, *et al*., (2022) used silver nanoparticles to inhibit *L. tropica*. In their study, several amounts of silver nanoparticles (2, 4, and 6 mg/ml) were examined. According to their results, the concentration of 6 mg/ml exhibited the maximum level of inhibition. On other hand, (Mohammed and Hussein, 2022) treat albino mice experimentally infected with cutaneous leishmaniasis. They used mixture of silver nanoparticles (AgNPS) and pentostam. Their results confirmed that the damaging effects of pentostam (Sb), silver nanoparticles (AgNPs), and loaded drug (AgNPs + Sb) on liver and spleen tissue were differed.

## Zinc oxide nanoparticles

Zinc oxide (ZnO) nanoparticles are zinc oxide (ZnO) particles with a diameter of fewer than 100 nanometers. They have important catalytic action and a substantial surface area in relative to their size. Zinc oxide nanoparticles' precise chemical and physical characteristics differ depending on how they are made. Antibacterial, anticancer, immunomodulatory, sunscreen, and antioxidant properties are just a few of the several healing uses for zinc oxide nanoparticles. They can also be active as an adjuvant to

diminish the damaging effects of chemotherapy medicines (Wisemann, *et al*., 2020). *In vitro* viability and growth rate of *L. donovani* promastigotes were examined by Enad and Zghair (2016) in relative to Zinc oxide nanoparticles (ZnO NPs) with a mean particle size of fewer than 100 nanometers (nm). In their investigation promastigotes growth rates and viability were assessed using the following ZnO NPs concentrations of (0.1, 0.2, 0.4, 0.6, 0.8, and 1 µg/ml) .They compared the activity of ZnO NPs with the same concentrations of pentostam. The inhibitory concentrations (IC50s) of ZnO NPs were noticed after 24, 48, and 72 hours, and the results showed significant differences between them. While the results of pentostam group showed that 50% of the parasites were viable in the same time interval (24, 48, and 72 hours). Zinc oxide nanoparticles (ZnO NPs) were also evaluated by Gharby and Al-Qhadi (2017) for their antileishmanial activity on the viability of *L. tropica in vitro* for both promastigote and amastigote stages. They evaluated the effects of several ZnO NP concentrations (2, 2.5, 3, 3.5, 4, 4.5, and 5µg/ml) on the viability of *L. tropica* promastigote compared to pentostam. According to their findings, the best cytotoxic effects of pentostam and ZnO were 40.81±1.47% and 25.12±1.47%, respectively, at high concentrations (5 µg/ml). The best concentrations of ZnO NPs on the viability of *L. tropica* promastigotes were determined by the IC50 based on the results of the MTT experiment. After 72 hours, the result was 4.318 µg/ml, while the pentostam showed an IC50 value of 4.897 µg/ml after 72 hours. Nonetheless, the study also confirmed how ZnO affected the amastigote phase, and that viability fell as concentrations and incubation times increased. So, after 72 hours, the highest concentration (5 µg/ml) revealed the lowest percentage of viability (18.17 ± 0.60 and 36.07 ± 2.68%) for both ZnO NPs

and pentostam, respectively. ZnO NPs and pentostam had IC50 values of 3.84 µg/ml and 4.734 µg/ml, respectively in the MTT assay results. Saleh (2018) examined how zinc oxide nanoparticles protected albino rats against *L.donovani* infection. The albino rats received 50ug/kg of ZnO-NPs for 14 days after receiving an injection of *L. donovani* at a concentration of 1.2× 1 0 6 cell/0.2ml. Glutathione (GSH) and malondialdehyde (MDA) evaluations were used to track the progression of *L. donovani* infection..

## Sodium chloride nanoparticles

Sodium chloride nanoparticles have the potential to be therapeutic agents with less adverse effects than existing treatments since they are toxic to malignant cells. It was stated that malignant cells are very hazardous to sodium chloride nanoparticles (SCNPs). This is an outcome of SCNPs' capacity to enter cells by endocytosis, which gets over cell restrictions on ion transport. SCNPs generate a spike in osmolality and rapid cell lysis when they dissolve inside cancer cells. Inquisitively, because of their comparatively low salt levels, normal cells are far more resistant to the treatment. SCNPs induce immunogenic cell death, or ICD, in contrast to traditional chemotherapeutics. Based on *in vivo* research, SCNPs have been shown to enhance anticancer immunity in addition to killing cancer cells (Jiang, *et al*., 2019). In a study conducted *in vitro*, Marhoon and Al-Musawi (2023) evaluated the influence of sodium chloride nanoparticles (NaCl NPs) versus the normal dose of Pentostam on the viability of *L. major* promastigote. NaCl NPs in different concentrations of 2, 4, 6, and 8 µg/ml were used. By cultivating the parasite in a cell culture microplate, these concentrations were observed *in vitro* on *L. major* growth. A new concentration of NaCl NPs was added after four days.

Far ahead, over the course of the four-day experiment, the number of promastigotes was counted every day using a hemocytometer stained with Trypan blue solution. Their findings revealed that when NaCl NPs concentration increased, the Growth Index (GI) rate of *L. major* promastigote reduced. For the concentrations listed, the Growth Index rates were 1.32×106, 1.31×106, 0.95×106, and 0.78×106. The rates of the Pentostam group and control group, which were 1.09×106 and 3.43×106, respectively, were compared with these values. Their findings indicated that the growth rate of *L. major* promastigote's decreased as NaCl NPs concentration rose.

## Conclusion

Most of these (*in vitro* and *in vivo*) experiments that used nano-particles in different concentrations succeeded in inhibiting the growth and viability of *Leishmania* parasites.

### Conflict of Interest

"The authors state that they have no conflicts of interest."

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