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# Nanoparticles as Developed Treatment Against Leishmaniasis: A Mini Review

**Lubna Abdulazeem**

DNA Research Center, University of Babylon, Iraq

**ABSTRACT:** External and internal parasites are the two types of parasites that are detrimental to human health. Parasites may live on the outside or within the host. Approximately 200,000 individuals are killed by parasite illnesses each year, which affect 3.5 billion people. In developing countries, these parasites cause fatal diseases such toxoplasmosis, leishmaniasis, trypanosomiasis, and malaria, which have high rates of morbidity and mortality. Endoparasites are organisms that live in biological environments and are a serious danger to human health, whereas ectoparasites are arthropods that transfer specific parasites or cause disease.

*Leishmania* sp. is one of the most important of these parasites. Leishmaniasis is caused by the obligate protozoan parasite *Leishmania*. Of the many parasite species discovered in this genus, twenty-one are considered pathogens. *Leishmania ethiopia*, *Leishmania donovani*, and *Leishmania amazonensis* are some of the species that cause leishmaniasis. Once an infected person or animal's blood is consumed by a sandfly, leishmaniasis is spread. The illness causes ulcers, and the infection can spread to other parts of the body. Visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucosal leishmaniasis (ML) are the three primary forms of leishmaniasis. Mucosal leishmaniasis symptoms often take one to five years to manifest. Most treatments for parasitic infections are no longer effective due to parasite resistance and a range of pharmacological side effects, even though many have been discontinued.

Chemical drugs and ethnobotanicals were used in the past to treat parasites. Resistance in parasites has been brought about by these chemotherapies. New biotechnologies that will enhance the safety of existing antiparasitic drugs and the accuracy, efficacy, and tolerability of diagnostic tests are thus the greatest means of addressing the problem of the aforementioned parasitic diseases. Chemical drugs and ethnobotanicals were used in the past to treat parasites. Resistance in parasites has been brought about by these chemotherapies. New biotechnologies that will enhance the safety of existing antiparasitic drugs and the accuracy, efficacy, and tolerability of diagnostic tests are thus the greatest means of addressing the problem of the aforementioned parasitic diseases.

In the discipline of nanotechnology (also known as "nanoparticle-based science"), physical, chemical, and biological systems with sizes ranging from submicrometer widths to individual atoms or molecules use technology. Research on pharmaceutical delivery and medical diagnostics are included. Pharmaceutical dosage forms based on nanotechnology are now being manufactured and used widely around the world. Advanced technologies in engineering, veterinary medicine, and medicine are only a few of the many different domains of knowledge that nanotechnology covers. New products based on nanotechnology, such as vaccines, recombinant proteins, and other pharmaceutical alternatives, provide safer environments for humans and/or animals. Biological applications such as drug delivery and issue engineering, bioimaging, and nanodiagnostics are among the many fields that have profited substantially from nanotechnology. Applications of nanodiagnostic methods for infectious diseases are growing in popularity due to their unique high sensitivity and early detection capabilities. Since antibiotic resistance is currently a threat to public health, several sectors have been established and significant progress has been made in the search for new and effective treatments. The development of antibacterial drugs heavily relies on nanomedicines because of their numerous advantages. Parasites, such as worms and protozoa, are common in Iraq. There have been a lot of studies done on diagnosis and treatment.

**KEYWORDS:** Leishmaniasis, Nanotechnology, antiparasites

## INTRODUCTION

There are several different *Leishmanian* obligate intracellular protozoan parasites that can cause a variety of clinical conditions that are grouped together under the umbrella term "leishmaniasis." Leishmaniasis is a tropical vector-borne disease (VBD) that is brought on by the *Leishmania* parasites and spreads to humans by the assault of female Psychodidae insects that feed on blood [1]. Visceral leishmaniasis (VL), mucocutaneous leishmaniasis (MCL), and cutaneous leishmaniasis (CL) are among the many illnesses caused by more than 20 different species of *Leishmania* [2].It is one of the top three parasite illnesses in the world, along with dengue fever and African trypanosomiasis, according to the Global Tropical Diseases Research Centre. The patients' diagnostic

complaints included anorexia, hepatosplenomegaly, headache, exhaustion, neutropenia, anaemia, and weight loss [3]. Specifically, *Leishmania donovani* is the infectious agent that causes visceral leishmaniasis. By entering their host cells through the cytoplasmic membrane, Leishmania infiltrates its hosts. Types of *L. donovani* include: The exterior, flagellated promastigote species and the internal, flagellum-less amastigote form are (i) and (ii), respectively [4].

It was long forgotten that leishmaniasis was a tropical illness. Its quick spread around the world, high risk, potential for death, and painful lesions in the tegumentary and visceral forms of the illness have made it a growing worry in every continent's healthcare system[5].

A set of parasitic diseases known as leishmaniasis are brought on by closely similar parasites of the genus *Leishmania*, which are members of the family Trypanosomatids and the order Kinetoplast Ida. Through their bites, infected phlebotomine sandflies spread the illness to animals[6]. These insects consume the blood of an affected person, which exposes them to the sickness. Vectors include more than 30 species of Lutzomyia and Phlebotomus [7]. Sandflies often exhibit their highest levels of activity in the early evening. Sandflies like to settle in damp, dark areas and are quiet, weaker flyers. Sandflies are more likely to infest adventure travellers, investigators working at night, construction workers, clergy, adventure tourists, and birdwatchers [8]. Leishmaniasis may manifest as a little skin lesion that goes away on its own, a crippling mucocutaneous disorder, or even a deadly systemic version of the illness [9].

Severe illness cases have always presented a significant therapeutic challenge, and the emergence of drug resistance only makes matters worse. According to the WHO, leishmaniasis is the sixth most prevalent infectious illness[10].

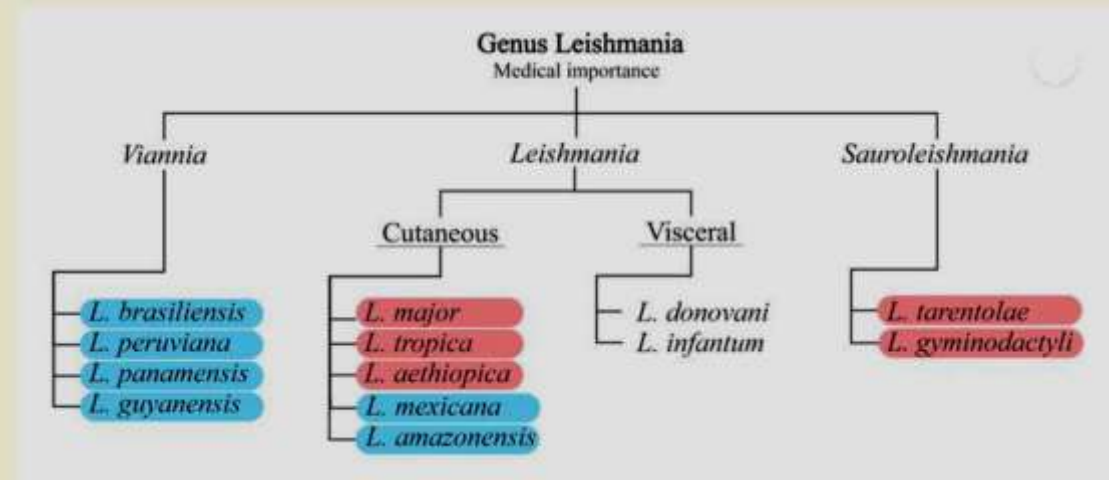


**Figure (2-1): An example of the three subgenera that make up the *Leishmania* genus. Here are a few of the species that have been the focus of the greatest scientific investigation. While amphibians are afflicted with Sauro leishmanial parasites, mammals are infected with parasites from the *Leishmania* and *Viannia* subgenus[11].**

**Structure of *Leishmania* sp.:**

The unicellular eukaryotic *Leishmania* species have a distinct nucleus and other cellular elements including flagella and kinetoplasts[12]. All *Leishmania* species have a single mitochondria, a single Golgi apparatus, and a single lysosome, each of which aids in parasite digestion by a unique enzymatic activity [13]. Two different structural forms exist, one for each stage of development.

**1- Amastigote:**

Mammalian mononuclear phagocytes and blood arteries include this form, which is stationary within cells due to the absence of external flagella [14]. Moreover, they are commonly found in endothelial cells, leukocytes, and monocytes. Osmotically absorb nutrients and utilise tissues as food. Instead of protruding from the cell's membrane, the tip of the small flagellum is embedded in it [15]. It is oval in shape, with dimensions of 3–6 µm for length and 1–3 µm for width. Near the front of the cell are the kinetoplast and basal body.(Figure 2., A) [16].

Along with vacuoles, the cytoplasm also includes blepharoplast and axonem. Giemsa staining turns the cytoplasm blue, whereas the nucleus is pink or deep red. Known as aerobes, they proliferate lengthwise inside [17].

**2- Promastigote:**

This is an extracellular, mobile kind that is housed in the digestive tract of a sand fly [18]. With measurements of 15-30 µm in length and 5 µm in breadth, it is notably longer and wider [19]. Both ends of the spindle-like item taper. The cell's lengthy flagellum protrudes from the front. The basal body and kinetoplast are positioned next to the nucleus, which is in the centre (Figure 2., B) [20]. The cytoplasm becomes blue when the Giemsa staining technique is used, while the nucleus stays pink or red.

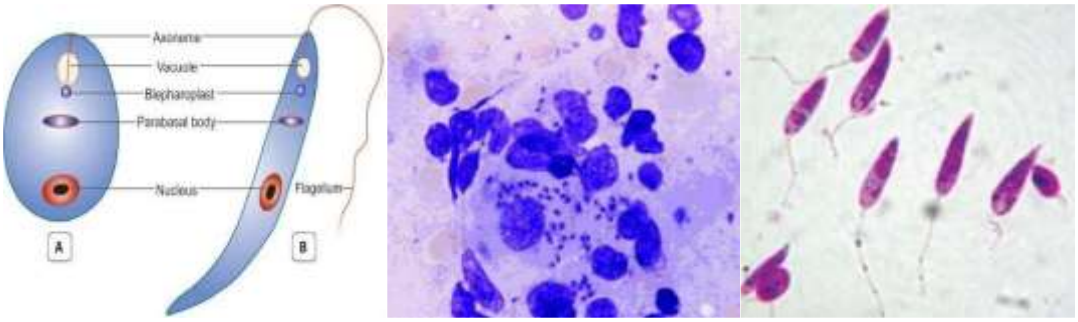


**Figure (2-2): *Leishmania*: a morphological scheme. (A): Amastigote, (B): Promastigote[21].**

***Leishmania donovani***

One of the hemo-flagellate kinetoplastids that causes leishmaniasis, *Leishmania donovani*, is an intracellular parasite that belongs to the genus *Leishmania* [22]. The infection of the mononuclear phagocyte system makes the spleen, liver, and bone tissue targets. *L. donovani* causes visceral leishmaniasis, which is commonly referred to as kala-azar [23]. Though it can occasionally linger on for a year or more, the incubation period typically lasts three to six months[24].

A kinetoplast, a flagellum, and a nucleus have all been identified in the single-celled parasite *L. donovani*. This species contains 36 chromosomes in total. It is host-dependent and comes in two structural types [25]. The amastigote form is common in the circulation and biological mononuclear phagocytes. On the contrary, the protozoan promastigote develops in the sandfly's digestive tract (Figure 4)[26].

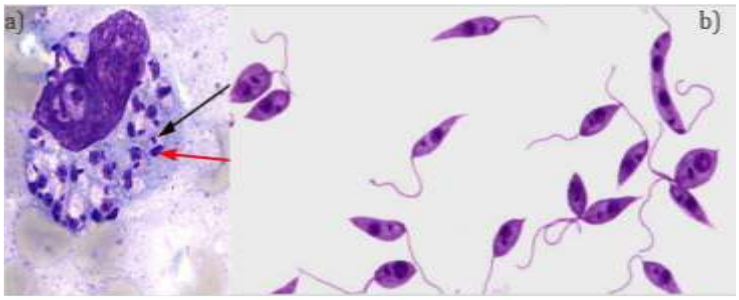


**Figure (2- 4): (a): Amastigotes form of *L. donovani* , (b): Promastigotes form of *L. donovani* [27].**

***Leishmania donovani* epidemiology:**

According to the World Health Organisation (WHO) (Global Leishmaniasis Surveillance: 2019–2020, a Baseline for the 2030 Roadmap.), *L. donovani* caused more than 13,000 visceral leishmaniasis cases in 2020. 200 million more people are at risk since more than 70 countries on all continents—aside from Antarctica and Australia—have confirmed instances of VL [28].

Despite being the most dangerous and instantly fatal form of *Leishmania*, *L. donovani* is the second most prevalent species that causes VL in humans. More than 90% of all instances of leishmaniasis that have been reported have occurred in Pakistan, Bangladesh, Nepal, Ethiopia, and Brazil. Even while endemic regions like Africa and South-East Asia have had fewer reported cases, the Eastern Mediterranean and European provinces are still considered vulnerable[29]. Medical experts in many nations have not given enough attention to VL caused by *L. donovani*, which has resulted in a lack of knowledge about the disease's occurrence, clinical symptoms, and early detection options. Between 2014 and 2019, the European Centre for Disease Prevention and Control (ECDC) tracked the incidence of VL caused by *L. donovani* in Europeans [30].

As per the results, the prevalence of infection has decreased considerably in Algeria, Morocco, and Tunisia while increasing in Azerbaijan, France, Greece, and Libya. Furthermore, ECDC (Surveillance, Prevention and Control of Leishmaniases in the European Union and Its Neighbouring Countries) reported new VL sub-nationally in historically non-endemic countries like Romania as well as epidemic countries like Italy, Greece, and Spain. Locally, Iraq would be a regional hotspot for leishmaniasis because to the prevalence of both VL (Kala-azar) and skin (Baghdad boil). After initially being reported in Mosul and Baghdad, leishmaniasis has subsequently spread throughout Iraq's regions [31]. Research by [32] indicates that this was the first time a figure this high had ever been discovered among Syrian migrants in Duhok. Iraq's growing urban population, growing poverty, and a general lack of care for people's living conditions might all be contributing factors to the prevalence of leishmaniasis, which has spread throughout most of the country's provinces [31].

***Leishmania donovani* life cycle**

For *Leishmania donovani* to finish its life cycle, it requires two distinct hosts: sand fly carriers as flagellated promastigotes and host human cells as intracellular amastigotes (Figure 5) [33].

Final host: While feeding on blood, the sandfly injects the promastigotes through the compromised person's epidermis. A bite from a sand fly releases parasites that are housed in its proboscis [34]. A small number of promastigotes may flow freely through the bloodstream before being cytolyzed by macrophages[35]. The phagocytosis process, however, involves the ingestion of several others by phagocytic cells found in the liver, spleen, and bone marrow. Amastigotes with a rounded shape are created by a temporary alteration within the cells [36]. Polymorphonuclear leukocytes can destroy promastigotes by oxidative means, but amastigotes are immune to this process. A mechanism known as simple binary fission is subsequently used by the surviving amastigotes to divide their cells [37]. Multiplication continues indefinitely until the host cell ruptures. When a cell is fully packed, 50–200 amastigotes are released into tissue compartments [38]. Following that, each amastigote may infiltrate fresh host cells. The entire tissue is gradually infected and destroyed as a result [39]. After entering the bloodstream, unbound amastigotes are mostly broken down by macrophages. Sandflies, which are blood-dependent, collect accessible and phagocytosed amastigotes from the outside of the body[40].

Intermediate host: *L. donovani* grows and matures only within the gastrointestinal tract of female sandflies. This makes females the primary carriers of the virus [41]. Amastigotes travel to the gut of the sandfly after being consumed. Chitin and protein are combined to form the peritrophic matrix, which covers the surfaces of the amastigotes. Thus, the parasites are protected from the digestive fluids of the host [42]. When the amastigotes reach the intestinal lumen of the belly, they undergo the first of several changes that result in their becoming weakly flagellated "procyclical promastigotes," mostly on the inner membrane[43].

The transformation into flagellar promastigotes causes them to significantly lengthen and expand. They stick to the intestinal epithelium and immediately split into two identical copies there [44]. Its membrane's lipophosphoglycan (LPG) makes it easier for promastigote attachment to the intestinal epithelium. These factors make it more difficult for the insect to expel promastigotes. Through genetic hybridization, they can further mate and produce sexual progeny in a sand fly's digestive tract [45]. The pharynx and buccal cavity, which are close to the mouth and the start of the digestive tract, are where they then return.

Only *Leishmania* exhibits the development of the anterior station [46]. After the initial blood feeding, a serious pharyngeal infection might appear six to nine days later. Promastigote secretory gel (PSG), which is composed of phosphoglycerate and aqueous acid phosphatase, is produced by them[47]. This time frame, referred to as the metacyclic phase, is when the promastigotes become contagious. Once inside the tubular proboscis, the metacyclic promastigotes group together until the feeding canal is completely closed. Soon after a bite, parasites enter the bloodstream of the human host and cause illness [48].

Reservoir host: Dogs are known to be susceptible to catching an illness brought on by *L. donovani*. In the centre of the Middle East, *domesticated dogs*, *Lycalopex vetulus*, and *Cerdocyon thous* (fox family animals) are susceptible to catching the zoonotic illness [49]. It is known that *L. donovani* is spread by a number of marsupials and mice in Brazil and Africa [50].

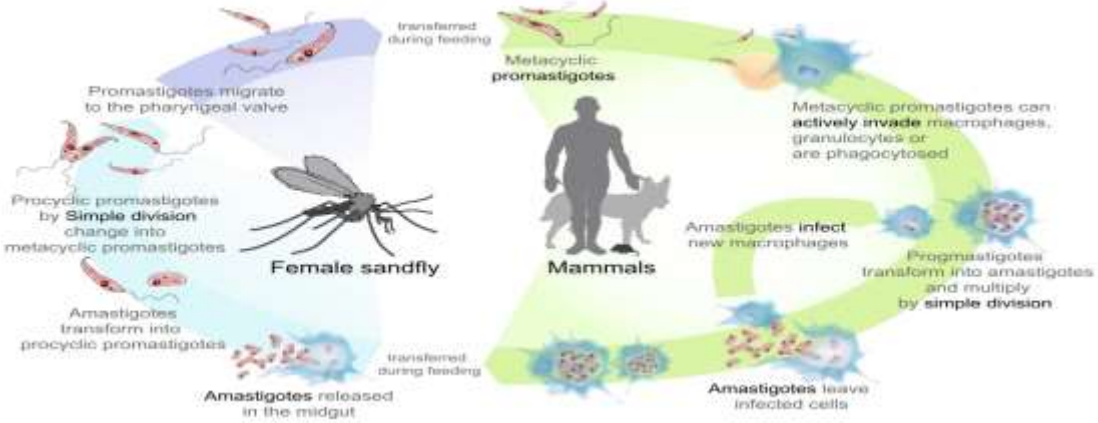


**Figure (2- 5): The stages of *Leishmania donovani* life cycle [33].**

**Nanoscience and Nanotechnology**

Originating from the Greek word for "dwarf" or "extremely small," the term "nano" is used to describe a measurement that is one thousand millionth of a metre (10-9 m) in size [51]. The production of novel biomaterials has increased dramatically over the last 30 to 40 years; nanomaterials in particular have found extensive application in a wide range of industries, including waste management, engineering, sports equipment, computers, textiles, and even food and personal care items[52]. The study of atomic, molecular, and macromolecule size manipulation of materials, structures, and technologies between 1 nm and 100 nm is known as nanoscience. The utilisation of such microscopic structures in technical domains such as electronics is sometimes referred to as nanotechnology[53]. Many people consider nanotechnology to be one of the most fascinating and potentially revolutionary scientific fields of the twenty-first century.

One of the most important steps in converting nanoscience theory into useful applications is the capacity to see, measure, manipulate, assemble, operate, and create materials at the nanoscale scale [54]. At the physicochemical level where biomaterial

interactions take place, nanoscale materials may be modified and designed to exhibit amazing features. Numerous medical applications are made possible by this, including the ability to identify biological markers in the early stages of a disease, target specific cells or tissues, create highly efficient drug delivery systems, and evaluate therapeutic approaches for degenerative illnesses [55].

**Silver nanoparticle (AgNPs) applications in parasitic diseases**

Because of their location inside cells, certain parasites are difficult to treat with antimicrobial drugs [56]. Therefore, it is imperative that new, safe, and effective anti-parasitic medications be developed, and that the transportation of current therapies be improved in order to increase their bioavailability[57]. Nano-delivery methods allow biological molecules to modify their shape, which improves their selectivity and sensitivity.

The possible antibacterial properties of silver, silver atoms, and silver-containing compounds have prompted a great deal of research [58]. Silver's antibacterial qualities inside microbial species are thought to be caused by the metal's interactions with soluble single-valent silver ions and catalysed processes[59]. In recent years, however, certain microorganisms have become resistant to the effects of silver compounds, which is comparable to resistance to common antibiotics. According to recent findings, nanoparticles have a useful antibacterial effect whether used alone or in composite materials [60]. Silver nanoparticles have been shown in several studies to be very effective at eliminating viruses and bacteria [61]. AgNPs have demonstrated exceptional disinfection properties, making them highly effective sterilising agents. [62].

A rapid diagnostic test (RDT) based on a histidine-targeted spectrophotometric biosensor was created employing AgNPs coated with nitrilotriacetic acid (NTA) to separate and purify histidine-tagged proteins. When it came to diagnosing P. falciparum histidine-rich protein (PfHRP-II), a critical marker of malaria in clinical specimens, this sensor exhibited improved specificity and acceptable heat persistence[63].

The most recent study used AgNPs as a SERS platform using label-free surface-enhanced Raman Spectroscopy (SERS) to detect *C. parvum* oocysts. This approach has been demonstrated to be a quick and efficient way to check water sources for this parasite, potentially averting outbreaks [64]. Trisodium citrate was utilized to chemically reduce silver nanoparticles throughout the preparation procedure.

Rodents infected with a strong *Toxoplasma gondii* (*T. gondii* RH) toxin were used to evaluate potential preventive and curative effects [65]. Additionally, AgNPs linked to tryptophan were examined for their antiparasitic effects in comparison to tachyzoites from related Toxoplasma species [66].

Silver nanoparticles were produced in an environmentally friendly manner using latex from Jatropha gossypifolia [67]. The phyto-synthesised silver nanoparticles (AgNPs) were combined with two microbial pigments, violacein and prodigiosin. Significant in vitro growth inhibition was seen in the chloroquine-resistant P. falciparum FcB1/Colombia strain[68].

When tested against *C. parvum* oocysts in culture after three hours of exposure, covalently organised AgNPs demonstrated antiprotozoal activity, with fatality rates of 47% and 73%, respectively [69]. Following their exposure to proteinate-encapsulated AgNPs in culture, *C. parvum* oocysts experienced significant distortion and a significant decrease in sporozoite excystation.

**Silver nanoparticles (AgNPs) as anti-Leishmaniasis**

The mitochondria and the nucleus are among the organelles that AgNP preferentially targets, according to several studies. There, it can interact with cellular molecules to potentially break down the inner membrane of the mitochondria, releasing reactive oxygen species (ROS) and breaking down AgNP to release Ag+ ions, both of which increase cytotoxicity[70]. Once silver nanoparticles were used to test the *L. amazonensis* parasite, apoptosis, reduced prevalence of infected macrophages, reactive oxygen species (ROS) formation, lack of mitochondrial stability, and disruption of the promastigote and amastigote phases were all observed[71].

However, the precise mechanism by which AgNPs carry out their antimicrobial effects is yet unknown. The suggested antiparasitic action of silver nanoparticles is depicted in figure 13 based on research data [72]. AgNPs can trigger the release of Ag+ ions, which can contaminate the cell walls and membranes of microbes.

(ROS) generated by Ag+ ions inside the cell are what induce the antiparasitic action, and they lead to the following series of events: repression of transcription and DNA replication, breakdown of biological membranes, leakage of cellular components, stimulation of protein synthesis and death, inhibition of cellular activity, mitochondrial degradation, and decreased electron transport network [73].

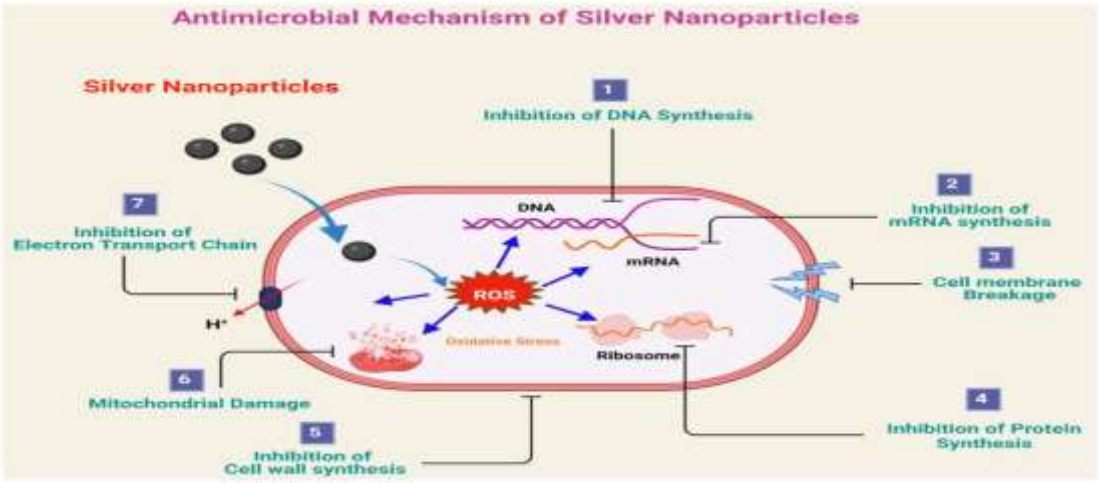


**Figure (2-13): Silver nanoparticles as antimicrobial [74].**

In vitro studies of chemically generated AgNPs examined their effects on the *L. tropica* MHOM/TR/99/EP39 strain's morphology, metabolism, multiplication, pathogenicity, and survivorship [75]. A range of AgNP dosages were examined, and the effects of each were examined under UV and in the dark. AgNPs changed the form and pathogenicity of promastigotes by preventing their growth and physiological processes. They also prevented amastigotes from being preserved in J774 macrophage cells during darkness. Importantly, exposure to UV light significantly increased the anti-leishmanial effects[76].

*Leishmania infantum* amastigotes in murine macrophages were eliminated at doses of 0.2-4 μM AgNPs, according to additional studies. Micromolar amounts of AgNPs that had been successfully captured by ferritin molecules in vitro increased the risk of mortality for both promastigotes and amastigotes [70].

To assess the effectiveness of a titanium dioxide (TiO2)-silver (Ag) nanocomposite against the parasites, promastigotes of *L. donovani* and *L. infantum* were employed. In the presence and absence of light, this nanocomposite had severely affected the parasites' physiological competence, including metabolic activities and survival[77].

Another work synthesized and characterized silver and titanium dioxide nanoparticles using a liquid extract of Euphorbia prostrata leaves. Each of the two metallic NPs was tested separately on *L. donovani* (strain MHOM/IN/80/DD8). Following a 24-hour in vitro interaction, bio-fabricated AgNPs showed the greatest level of effectiveness against parasite stages, suppressing promastigotes and amastigotes by 50%[78].

Numerous investigations employed fluid leaf preparations from three plants (soybean, coriander, and fenugreek) to produce bioactive Au-Ag NPs (gold-silver bimetallic NPs) by a conventional reduction process. After these NPs were administered for 48 hours, the quantity of intracellular amastigotes was greatly decreased, and *L. donovani* promastigotes experienced apoptosis[79].

The key component of a novel method to reduce and encapsulate silver metal to different nano-forms was the anti-leishmanial chemical 4',7-dihydroxyflavone (47DHF). Tyrosine aminotransferase is one of the well-known enzymes found in *L. donovani*, and they found that this chemical inhibits it. Furthermore, the biological mechanism and efficacy of silver nanoparticles against *leishmania* are evaluated. According to the study's findings, 47DHF-coated silver (Ag-47DHF) has the potential to be a potent anti-leishmanial agent [80]. AgNPs were produced using herbal extracts, which reduced metal cytotoxicity, according to [70]. The comparator drug miltefosine had IC50 levels that were substantially higher than those of the AgNPs generated against promastigotes. AgNPs were useful and comparable to miltefosine in terms of causing apoptosis, producing internal ROS, and reducing amastigote survival[81].

Earlier research by Bagirova *et al*. synthesized AgNPs using Cuminum cyminum seed extraction and compared their leishmanicidal efficacy to that of green-produced AgNPs. Furthermore, the biologically synthesized AgNPs were more effective (80%) and safer (12%) against *L. tropica* and *L. donovani* than pure AgNPs, which had a 2% biocompatibility.Unfortunately, this study did not contain an in vivo leishmanicidal evaluation [82-84].

## CONCLUSION

This study looked at leishmaniasis therapy from a variety of perspectives as a worldwide disease. Despite the rapid global expansion of parasite diseases, comparatively little study has been conducted on their treatment. This carelessness has led to a subpar and harmful therapy process continuing. To solve this problem, it is crucial to use medicine delivery methods that increase therapeutic efficacy while reducing negative effects. Research has been done on the use of nanotechnology to cure leishmaniasis. Using nanoparticles, a revolutionary drug delivery technique that improves sensitivity and lessens side effects, anticoagulant drugs may be administered to certain cells.

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