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# Therapeutic Potential of Nanoparticulate System for Treatment of Protozoan Infection

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## Abstract

Protozoan parasites cause serious human and zoonotic infections, including life-threatening diseases such as malaria, African and American trypanosomiasis and Leishmaniasis. According to World health organization evaluation, these diseases are no more common in the developed world, but together they threaten about 40% of the world population. Mortality and morbidity are elevated in developing countries, and lack of vaccines makes chemotherapy the only option. However, available anti-parasitic drugs are hampered by more or less marked toxic side effects and by the emergence of drug resistance. Among all protozoan diseases, Leishmaniasis is prevalent and is one of the most neglected tropical diseases in terms of drug discovery and development. Recent improvements have been accomplished by combination of nanoparticle mediated therapy. The use of nanoparticulate system for the delivery of therapeutic agents is receiving considerable attention for medical and pharmaceutical applications. The establishment of new approaches related to nanoparticle technology allowed the identification of new molecular targets that in some cases are shared by different parasites. In this article, we summarize the nanotechnology based current development in the leishmaniasis disease which can cross host or parasite natural barriers and, by selectively delivering new or already in use drugs to the target site, minimize

**Keywords:** Leishmaniasis; Visceral Leishmaniasis; Nanotechnology; Nanomedicine; Phytodrugs; Drug Delivery;

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## Introduction

Protozoa are unicellular eukaryotic microorganisms. Morphologically they are of various forms and structure and their physiology and metabolism are adapted to their needs. Mostly, they live as parasitic forms but they do exist as vegetative (trophozite) and resistant (cyst) forms. Parasitic forms are vector-borne protozoan's and are of public health concern in the tropics includes Sporozoa, Rhizopoda, ciliates, and Flagellates. Diseases caused by Plasmodia (malaria) and trypanosomiasis (leishmanaisis, African human trypasnosomiasis and chagas) represent a major public health concern in the tropics. Discussion and analysis on the major and new approaches and strategies currently employed in an effort to minimize the burden of leishmaniasis diseases and the major advancement occur in this field globally is the concern of this article.

**Leishmaniasis**: is a disease caused by an intracellular protozoan parasite (genus *Leishmania*) transmitted by the bite of sandfly. The life cycles of the *Leishmania* spp. are relatively simple, involving a mammalian host and a vector stage (Figure 1).

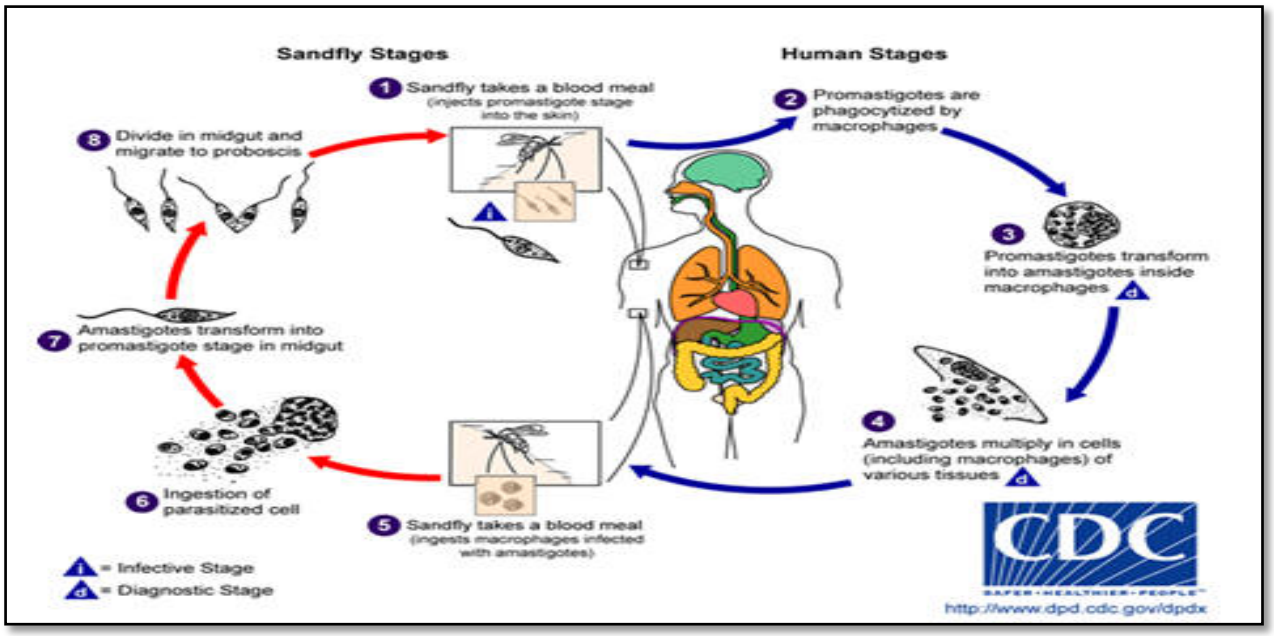


**Figure 1:** Schematic diagram of the *Leishmania* digenetic life cycle. From: http://www.cdc.gov/parasites/leishmaniasis/biology.html

This genus *Leishmania* includes a great number of species, among which are *L. donovani*, *L. tropica*, *L. major*, *L. infantum*, *L. braziliensis*, with a biological cycle integrated by an extracellular form or promastigote in the intestinal tract of phlebotomes and an intracellular form or amastigotes that mainly attack the cells of the reticulo-endothelial system. Promastigotes are quickly phagocytized by resident phagocytes, transformed into tissue-stage amastigotes, and divide through simple division in parasitophorous vacuole. The clinical spectrum of leishmaniasis ranges from a self-resolving cutaneous ulcer to a multiating mucocutaneous disease, even to a lethal systemic illness at viscerotropic levels and post-kala azar dermal leishmaniasis (PKDL) (Figure 2).

Therapy has long been challenge in the more severe forms of the disease, and it is made more difficult by the emergence of drug resistance. In the absence of treatment, the case fatality rate of fully manifest clinical visceral leishmaniasis (VL) without treatment is greater than 90%. Leishmaniasis is endemic

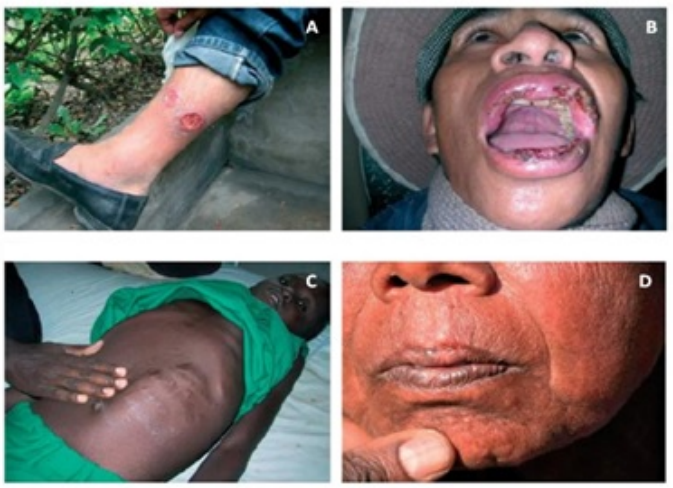


**Figure 2:** Different clinical forms of Leishmaniasis: (A) Cutaneous Leishmaniasis, (B) Muco-cutaneous Leishmaniasis, (C) Visceral Leishmaniasis, (D) Post Kala Azar Dermal Leishmaniasis.
From:http://medicscientist.com/leishmaniasis-kala-azar-treatment

in 98 countries and 3 territories, concern regions are in Asia, Africa, Central and South America and the Mediterranean area of Europe [1]. Majority of cases occurring in developing nations. More than 90% of VL cases occur in India, Bangladesh, Sudan, South Sudan, Ethiopia and Brazil (Figure 3); moreover 50% of the global burden of this neglected disease [2]. The distribution of competent vector species and leishmaniasis has prolonged over the last decade, probably due to an elevation in an acquiescent environment of vector species due to shifts in climatic conditions [3]. Clinical VL has also been associated with poor nutrition [4]. Further risk factors are related to their immunologic status and their ability to clear infection or maintain an asymptomatic state. These factors include concurrent infection with Human Immunodeficiency Virus (HIV), drug abuse and other immune suppressions. Further the emergence of VL-HIV co-infection has enabled this disease to expand its geographical range. To date, *Leishmania* and HIV co-infection has been reported in 35 countries, with most of the cases from southern Europe, where 25–70% of adult patients with VL are co-infected with HIV, and 1·5–9·0% of patients with AIDS develop leishmaniasis [2]. The fundamental prevention of leishmaniasis requires the blocking of a step in the parasite's life cycle. The current method of VL diagnosis evaluating clinical symptoms that include fever for more than two week, the presence of splenomegaly, and a positive serological rK39 immunochromatographic rapid diagnostic test (RDT) [5, 6].

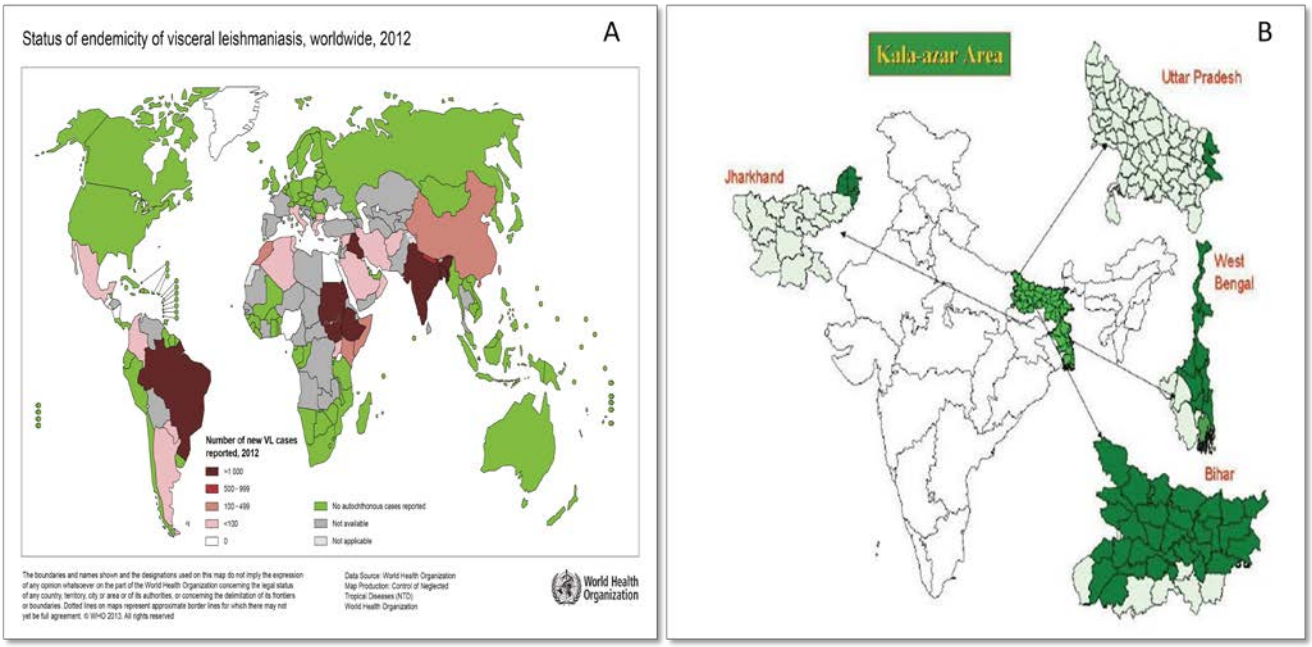


**Figure 3**: Endemicity of Visceral Leishmaniasis in (A) World and (B) India.

A. http://gamapserver.who.int/mapLibrary/Files/Maps/Leishmaniasis_VL_2013.png?ua=1

B. http://www.indg.in/health/diseases/kala-azar

## Therapeutic modalities, Phytodrugs and their limitations

Pentavalent antimonials (SbV) are first line drug accessible for the treatment of Leishmaniasis since nineteen's. Amphotericin B and Pentamidine are considered as second line drug for the treatment of VL. Drug selection is related to the causative *Leishmania* species. More recent drugs such as Miltefosine (an oral drug), Paramomycin and Sitamaquine have shown significant efficacy against VL. The major problems associated with conventional therapies such as high toxicity, growing rate

of parasite resistance to current drugs and due to prolonged use of drug leads to severe side effects along with the expensive cost of medication make them poor tolerated drug [Table 1].

**Table 1**: Summary of Characteristic of antileishmanial drugs in current chemotherapeutics

| S. No | Drugs | Regimen | Brand Name | Mechanism of Action | Limitations |
|---|---|---|---|---|---|
| **1** | Pentavalent antimonials | 20 mg/kg daily for 20-40 days depending upon geographical area, intravenously administrated | Pentostam, Stibanate, Generic sodium Stiboglucanate | Action on macrophages, activated within the amastigote form | Resistance, toxicity in HIV co-infection, higher costs, cardio and renal toxicity, vomiting and abdominal pain on long term administration |
| **2** | Amphotericin B | 7-20 mg/kg total dose for up to 20 days, intravenously administered | Fungizone | Binds with the ergosterols of the parasitic cell membranes thus forming a binary complex with the membrane sterols resulting in pores which causes changes in membrane permeability and ionic balance leading to parasitic cell death | current second line when antimonials not appropriate, Dose limiting toxicity, poor gastro-intestinal absorption and negligible bioavailability, also may react with mammalian cell membrane causing cellular dysfunction |
| **3** | Lipid associated amphotericin B | 10-20 mg/kg total dose in 5-10 doses over 10 days, depending on geographical area, intravenously administered | Ambisome | Same as Amphotericin B | Cost and cost effective, reportedly more effective and slightly toxic |
| **4** | Colloidal dispersion | 10-15 mg/kg total dose over 5 days | Amphocil | | Optimum doses need to be defined |
| **5** | Pentamidine isethionate | 4 mg/kg on alternate days or three times per week for about 15-30 doses, intravenous or intramuscularly administered | | Binds to tRNA and inhibits aminoacylation and translation of the replicating parasite | current alternative second line drug, increasing unresponsiveness in India, cardiac arrhythmia, acute renal failure, hypotension |

| | | | | | |
|---|---|---|---|---|---|
| **6** | Miltefosine | 2-5 mg/kg total twice dose up to 28 days orally administered | | Mechanism of action is uncertain, possible inhibition by phosphatidylcholine biosynthesis, signal transduction and regulation of calcium homeostasis | Adverse effects includes gastrointestinal disturbance and renal toxicity, teratogenic |
| **7** | Paromomycin | 15-20 mg/kg daily for about 21 days, intravenous or intramuscular administered | | Impairs the macromolecular synthesis and alters the membrane properties of Leishmania | Mainly used in the cutaneous form of the disease and has limited use in the treatment of VL |
| **8** | Sitamaquine | 1.75-2 mg/kg/day for 28 days in India | | unknown, possibly affects mitochondrial electron transport chain | |

The problems of developing a single drug or formulation for all forms of leishmaniasis revolve around factors that includes different sites of infection impose the need of drugs with varying pharmacokinetic properties, different *Leishmania* species exhibits variations in drug sensitivity. Various studies for achievement of a vaccine for leishmaniasis immunization are accomplished since early nineteen's [7]. The vaccine prepared against leishmaniasis are comprises of vaccines based on dead parasite, attenuated parasites, antigenic fragments and genetic vaccines based on DNA [8]. First generation killed parasites demonstrated limited efficacy in phase III trials moreover well defined molecules has not reached to phase III yet. Limited efficacy in vaccines against leishmaniasis is partly due to lack of an appropriate adjuvant. In few countries some clinical trials were performed but none of the vaccines showed a level of efficiency higher than 80%, a fact that makes unfeasible human test. Due to intense collateral effects, lots of patients who infected with leishmaniasis refuse treatment, and there is the need to new alternative treatments. Hence particulate based delivery system appears very promising to solve all the limitation of available drugs, chemotherapeutics and vaccines. The drug discovery against leishmaniasis has been more capable on therapeutic switching rather than discovery of novel therapeutic drug candidates. Among natural resources, plants based products have been most extensively explored for bioactive antileishmanial efficacy. Various herbal formulations and plant secondary metabolities such as alkaloids, tannins, flavonoids etc are known to be effective against leishmaniasis treatment but only limitation is their poor bioavailability and efficient delivery to cells. Thus, conventional treatment of *Leishmania* faced with many serious problems, leading in enormous efforts to develop new therapeutic strategies among them are phytotherapy, synthetic and or semisynthetic medication with botanical and medicinal basis and improved pharmacological approaches to transfer drugs directly into the cells through nanoparticulate delivery system. Nanoparticle based delivery system aid the transfer of leishmanicidal agents

that invade to intracellular parasites with least toxicity and higher efficacy [9].

## Nanotechnology

Nanomedicine is the medical application of nanotechnology. Nanomedical approaches to drug delivery center on developing nanoscale particles [9]. Nanotechnology can be useful tool for formulating new drugs against leishmaniasis. Nanoparticles like emulsion, liposome, nanospheres, polymeric and metallic particles have been of great importance for drug delivery as drug carrier [9]. Already present drug as well as phytodrugs is now introduced with the aid of nanoparticulate compounds against leishmaniasis [10 - 13]. Broadly, two strategies may be employed for delivering antileishmanial drugs to infected site i.e. passive drug delivery and active drug delivery. Passive drug delivery utilizes the use of conventional nanocarriers i.e. biodegradable polymers, liposomes, metallic nanoparticles without surface modification [14], while active drug delivery of cell specific therapeutic drugs associated with nanocarriers is achieved by attaching a cell specific ligand at the surface of a carrier, thereby allowing the accumulation of drug in targeted cell [15]. Nanodrugs that have been used in the treatment of leishmaniasis have exhibited greater efficacy than free drugs that may be attributed to efficient uptake by the cells than micromolecules. The summary of main drug delivery system against leishmaniasis is given in table [Table 2].

**Table 2**: Summary of main drug delivery system against Leishmaniasis

| S. No. | Delivery System | Drugs/Phytodrugs/Antigen | Parasite | References |
|---|---|---|---|---|
| **1** | Liposomes | Antimonials | *L. donovani, L. major* | 16 |
| | | Andrographoid | *L. donovani* | 17 |
| | | Pentamidine | *L. donovani* | 18 |
| | | Miltefosine | *L. donovani* | 19 |
| | | Amphotericin B | *L. donovani* | 20 |
| **2** | Polymeric particles | | | |
| | Starch-Microparticles | Antimonials | *L. donovani* | 21 |
| | PLGA Nanoparticles | Amphotericin B | *L. donovani* | 22 |
| | Polycaprolactone | Amphotericin B | *L. donovani* | 12 |
| | Nanoparticles | | | |
| | Nanosuspension | Amphotericin B | *L. donovani* | 11 |

## Conclusion

Pharmaceutical research on nanotechnology mediated drug delivery system represents a major strategy for discovering and developing new drugs against leishmaniasis. Current chemotherapy used in the treatment of leishmaniasis is limited by its toxicity. Nanoparticulate technology endows an indefinite opportunity for improving the efficacy and reducing toxicity of presently accessible antileishmanial drugs. The therapeutic advantages of nanotechnology mediated drug delivery are becoming perceptible and may soon be associated with every route of administration. The targeted drug delivery system offer the facility of administration of drugs at lower doses with targeted specificity, if safely formulated. Nanoparticles may be formulated to serve as efficient carrier for drugs or antigens/adjuvant as vaccine, of interest. Though, nanotechnology has potential future but it is important to consider the toxicological aspects of nanoparticles in both *in*

*vitro* and *in vivo* models to assure that these materials will not persuade any cellular reaction in human body. Much of research needs to be done in the field of nanotechnology based drug delivery so as to curb the deadly disease leishmaniasis. Nanotechnology that employs the use of nanoparticles as carriers of the drug has provided some hope for the elimination of leishmaniasis. Nanotechnology has shown potential to prepare delivery system of greater efficacy for treating and preventing leishmaniasis. The application of nanotechnology for the treatment of leishmaniasis has shown promising results which could result in the culmination of the disease. Thus the drug delivery using nanoparticles is the need of the day.

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