



Current Strategies and Advances in Nano Systems for Management of Leishmaniasis

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ABSTRACT

Leishmaniasis is a deadly parasitic disease transmitted by phlebotomines and flies. It is a neglected tropical disease, especially concentrated in the less developed countries; leishmanial infection is responsible for high molarity rate. The infection is classified as three major form: mucosal (ML), cutaneous (CL), and visceral leishmaniasis (VL). The nature of intracellular and disseminated location of parasite, the chemotherapeutic agents are very limited, and increasing the resistance of first line drugs creates a great challenge to formulation scientist that have to make necessary effective management of this Leishmaniasis infection by altering the delivery of resisted (or) existing drugs. Over the past decades, research on development of modulated treatments such as novel based drug delivery system (nanotube, nano suspension, nanoparticle, liposomes etc.); the use of anti leishmanial drugs, development of anti leishmanial vaccine as well as natural products has been extensively investigated.

Keywords: Leishmaniasis; Liposomes; Nanoparticles; Natural products; Vaccine

INTRODUCTION

The leishmaniasis is a ignored vector-borne deadly tropical disease; this infection is caused by intracellular protozoan parasites it belongs to genus *leishmania*, and the most severe type of infection transmitted by the bite of female *phlebotomines* and-flies of genera *lutzomyia*, family: *psychodidea*; order: *Trypanosomatidea* [1-4]. This leishmanial infection is further classified as mucosal (ML), cutaneous (CL), and visceral leishmaniasis (VL), which is also commonly named as kala-azar. This infection as a potentially lethal and neglected disease; it is presently a major cause of the developing countries. The World Health organization (WHO) recognizes this leishmanial infection which is one of the most severe and uncontrollable disease it shows a major health problem international. The mortality rate of leishmaniasis is so high, with about 400000 people and nearly 30000- 40000 deaths each year in about 98 countries which spread over 4 continents except Oceania and Antarctica. The distribution is seen high in tropical and subtropical regions mainly of central and South America, Africa and Southeast Asia due to the more prevalence of sand-fly vectors [5]. The common form of infectious disease is CL, its symptoms are skin sore at the bite site, and this infection heals in a 5-10 months but it leaves an unpleasant- looking scar whereas ML starts with skin ulcer which spreads and causes damage and destruction of the tissue. VL is also known as kala-azar, this infection considers as most deadly disease is characterized by loss of weight, progressive swelling in the spleen and liver, anemia, irregular bouts of fever, and severe fatigue [6]. The life cycle of leishmanial infection is sketched in Figure 1. The new cases of leishmanial infection about 0.3 million VL and 1.0 million CL are reported annually and 12 million CL are being infected with >340 million are highly prone of developing the leishmaniasis disease [7-10]. The leishmaniasis management is more difficult when compare to other parasitic infection due to rapid proliferation caused due to microphages invasion and rapid differentiation forming a mass called amastigotes which finally kills

the post mononuclear phagocytic system. This kind of invasion of parasite into the post immune system alters the membrane permeability of microbial activity. Therefore treating the leishmanial parasites which have invaded, the host is treated by using high molecular specific targeted therapeutic agent [11].

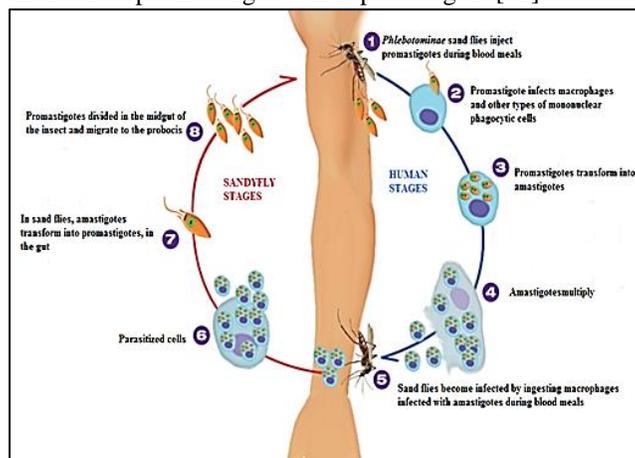


Figure 1: Life cycle - leishmaniasis

Leishmania Parasite Life Cycle

The primary learning of life cycle of *leishmania* parasite can be understood by the various different stages. It undergoes which help in providing ideas for planning the proper treatment of leishmanial infection. The vectors which are basically believed to be different species of the sand-fly (genus *lutomyia* or *phlebotomus*). A protein needed by the female sand-fly during the egg laying period take a blood meal of humans or domestic animals thus leads to transition of leishmanial parasite. The life cycle of genus leishmanial a protozoan parasite alternates between two forms, namely promastigote (flagellar) form and amastigote (replicative) form (Figure 1). The promastigote found in the midgut of sand-fly vector (the motile elongated 10-20 μm flagellated form) and amastigote (oval 3-7 μm non-motile, non-flagellated) which mainly reside in humans (macrophages) and other vertebrate hosts [12]. When the sand-fly vector sucks the amastigote infected blood from host, these amastigote convert to promastigote form within five hours of ingestion inside the gut of the insect. Here in the duration of 24-48 hours, the amastigote present completely convert into actively motile promastigote by the process of binary division. Within the term of 6-9 days of ingestion of infected blood the promastigote moves to sand-flies mid-gut, and when this sand-fly bites a new host gets infected with promastigote. Which lead to rapid infiltration of macrophages and neutrophils present at the bite site, where the promastigote becomes immotile and converts back to amastigote form? These amastigote infiltrate and take shelter in the cells of reticuloendothelial system (RES), it is at this place they undergo multiplication by binary fusion. When this RES cells gets ruptured, it leads to liberation of 40-180 amastigotes and causes disruption of the immune response by the T-Helper cell type 1 gets impaired inputs leading to migration of these parasite into the circulation to raid fresh cells for further proliferation [13]. Alvar reported as [14] the various classes of leishmaniasis are prominent in different regions of the world. The 90% of world's VL cases are being endemic in Nepal, Bangladesh and India. The 95% of world's CL cases are being endemic in Iran, Columbia, Afghanistan, and Algeria. The 90% of world's ML cases are being with endemic in Brazil, Bolivia, and Peru [15- 18].

Pathophysiology

Leishmania parasites can successfully develop in both vertebrate and invertebrate hosts due its digenetic nature of life cycle. The main two morphological forms have been recognized in its life cycle; namely promastigote form found in the vector, and amastigote form that preferentially resides in macrophages and other phagocytic cells. Promastigotes are motile, elongated (10-20 μm) and flagellated form of the parasites that undergo differentiation process (metacyclogenesis) ultimately resulting in metacyclic form of the parasite, which is highly infectious and non-dividing in nature. Contrary to promastigotes, amastigotes are oval (3-7 μm in diameter), non-motile and non-flagellated in nature, with additional capability to develop and replicate in the phagolysosome of the host mononuclear phagocytes. Acidic pH, hydrolytic enzymes as well as reactive species of either nitrogen or oxygen are incapable to retard its survival and replication; which is unusual when compared to other pathogens [19,20]. Infection to host is spread during blood feeding by *Phlebotominae* and fly transmitting metacyclic promastigotes which rapidly are internalized by the phagocytic cells (dendritic cells and neutrophils, macrophages). This

transmission occurs either directly or indirectly where the neutrophils assemble at the bite site of sand-flies and causes infection by promastigotes, leading to spontaneous apoptosis and allowing indirect entry of parasites into their host cells (Trojan Horse Model) [21]. Promastigotes accumulate and reside in phagosomes of macrophages which bind with lysosome developing the phagolysosome, where promastigotes differentiates into amastigotes which is further facilitated by variance in temperature in sand fly 25°C to in host 37°C and acidic environment in phagolysosome. Amastigotes further proliferate in the host cells *via* binary cell division, which ultimately leads to its lysis and allowing the infection to spread to other macrophages, including phagocytic cells and non-phagocytic cells like fibroblasts. Sand-fly takes up these infected macrophages along with blood meal initiating the later stages of parasitic life cycle. At this stage, the amastigotes convert to flagellated and slightly motile procyclic promastigotes that multiply by binary fission. During the later stages of metacyclogenesis, the procyclic amastigotes differentiate to infective and non-dividing metacyclic promastigotes, which *via* the salivary glands of sand-fly are capable to infect the mammalian host through lipophosphoglycan and glycoprotein 63 virulence factors. Among the numerous reported *Leishmania* species (~30), around >21 seem to be pathogenic to humans [22,23].

Diagnosis

The common symptoms of VL are found to be irregular fever, leucopenia, anemia, and hepatosplenomegaly. Various methods of laboratory diagnosis of leishmanial infection are used in which the current diagnosis includes parasite detection by microscopic examination culture and succeeding iso-enzyme analysis for identification of the parasite or spotting the parasite DNA (polymerase chain reaction [PCR]) present is detected by molecular biology based assay [24]. Which are highly sensitive and carried out at only limited centers. In recent study the PCR method holds and upper hand on sensitivity when compared to the microscopic smear [25-27]. Various other test namely immunofluorescence antibody assay (IFA), enzyme-linked immunosorbent assay (ELISA), western blot technique or leishmania antigen detection in urine by latex agglutination. The diagnostic antigen namely rK26 and rK18 have been shown suitable as an indirect measure of parasitic clearance. ELISA test carried out with the use of recombinant *L. infantum* heat shock protein 83 (rHsp83) helps in conformation of presence of CL and VL. Integrated diagnosis based on the combination test of rK39 rapid test and molecular test, (ELISA, PCR) are helpful in diagnostic assessment of patients [28-30].

Current Advances in the Treatment of Leishmaniasis

The combination of pentavalent Paramomycin and antimonial is prescribed for leishmanial disease was established in the year 1945, and was priority treatment for VL and CL disease in large number in the world. Pentamidine and AmB are second-line drugs for the management of leishmanial infection, even though long term administration of parenteral route is necessary. The alternative drugs for treatment of leishmaniasis relays on causative species of leishmania [31].

Parenteral Drugs

Sodium stibogluconate:

The initial dose of this drug was used 10 mg/kg for 6-10 days. The last five years have seen the resistance of the sodium stibogluconate is emergence of large scale. The current recommendation is replacement of sodium stibogluconate by amphotericin B [32] (Figure 2).

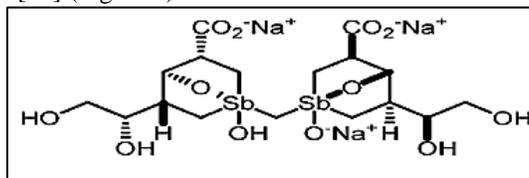


Figure 2: Sodium stibogluconate

Pentamidineisethionate:

Pentamidineisethionate is being used as a second-line medicine for management of VL but this drug results specific mode of action has to be explained. Hence it is a competitive inhibitor of arginine transport and non-competitively inhibits spermidine and putrescence. This drug was used initially confirmed to be useful in sodium stibogluconate resistance in India [33] (Figure 3).

Amphotericin B (AmB):

AmB is a drug it shows macrolide antifungal antibiotic activity, this drug isolated and developed from the species of *streptomyces nodosus*. It is a super leishmanicidal activity. The dose of amphotericin B is 0.75 to 1 mg/kg for 15 to

20 in fusions daily. The main preventive factors contain the occurrence of infusion based reaction such as high fever rigor chills, renal dysfunction, and even death. This AmB drug cause's high level toxic to the patients [34] (Figure 4).

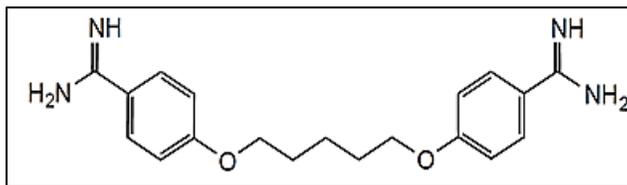


Figure 3: Pentamidineisethionate

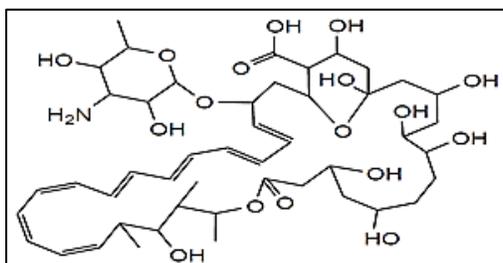


Figure 4: Amphotericin B

Oral Chemotherapeutic Agents

Miltefosine:

It is an alkylphospholipid derivative and it's originally registered for antineoplastic activities. The miltefosine is first-line oral drug for treatment of leishmaniasis. The dose of the drug is 100 to 150 mg for 28 days. The both human and animal clinical study report shows various adverse effect of miltefosine drug such as vomiting, diarrhoea, and include gastrointestinal disturbance (Figure 5).

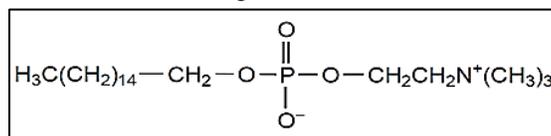


Figure 5: Miltefosine

Paromomycin:

The Paromomycin is isolated from the cultures of *Streptomycesrimosus*, these anti-leishmanial drug classified under category of aminocyclitol aminoglycosides and this drug prescribed both anti-protozoal and anti-bacterial activity it has been used either alone (or) compound with (sb^v) for treatment of VL [35] (Figure 6).

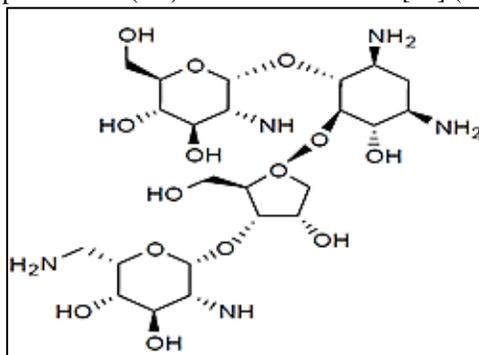


Figure 6: Paromomycin

Other Oral Compound

Azoles: The azoles derivatives are essentially sterol bio-synthesis inhibitors and specifically block ergo sterol synthesis.

Drug Sensitivity and Drug Resistance

The drug resistance and sensitivity of drug is the major obstacle in effective leishmanial chemotherapy. Paramomycin and miltefosine is the orally administrable first-line drug for leishmaniasis, these drugs are results sensitivity and resistant to the cultures of *Leishmania*. At the same time these sensitivity and resistance parasite of oral first-line anti leishmanial drug in *in vitro* laboratory studies create an aware about efficient use and life span of anti leishmaniasis drugs [36-38]. The resistant parasitebacteria contain major mechanisms to reduce anti-bacterial activity of anti-leishmanial drugs, such as increasing drug efflux, altering the chemical properties of drug, and increase cell wall protection. In via, due to the long half-life of drug around 140 hour sit may cause the development of resistance to the bacteria or drug [39]. The treatment of leishmanial infection is more complicated by different inefficacy of the drug.

Intrinsic deviations in the sensitivity of drugs:

The sensitivity to miltefosine, azols, paromomycin, and antimonials contain at least 3-4 fold deviations among the *leishmanial* specious.

Drug resistance development:

Leishmanial tropica, and *leishmanial dodovani* had a great drug resistant to the public, in India 60% of VL patients suffers because of pentavalentantimonoal drugs.

Immune status:

Absence of T-cell leads to decrease the antimonials activity; it cases the absence of immune response has impact in management of CL and VL.

Current Strategies in the Treatment of Leishmaniasis**Nanotechnology:**

Since few past decades, nanotechnology have been focused much concentration towards its application to pharmaceutical field. This technology can be defined as a nano sized colloidal system composed of natural or synthetic or semi-synthetic polymers. The main aim of nanotechnology to overcome the major problems in the conventional dosage form, such as poor bioavailability, undesirable side effect, and limited effectiveness, it also increases the drug concentration in specific target site. The current research of nanotechnology has proved that nanoparticle acquire a better potential as conventional dosage form. Usage of nanoparticles is swiftly emerging technology that can be chosen as important tool for targeted drug therapy [40]. In 1980 K. Eric Drexler developed and promoted the concept of nanotechnology. The notable characteristic of each of this nano system is their diverse physicochemical properties and discrete size range in nanoscale. Example nanoparticle (10-1000 nm), liposomes (80-200 nm), gold nanoparticles (5-50 nm), polymer therapeutics (5-25 nm), block copolymer micelles (50-200 nm), and nanosized crystals (10-1000 nm). This nanosystem can be incorporated for therapeutic purposes in order to deliver the dosage form from site of administration to the target site in a desired format. The pharmaceutical research has been focused in development of these nanosystem and some of different classes of nanosystems are plotted in Figure 7. The nano carrier being a radical approach are at forefront of the rapid development field of nanotechnology and have wide scope of applications in drug delivery, clinical research and medicine [41].

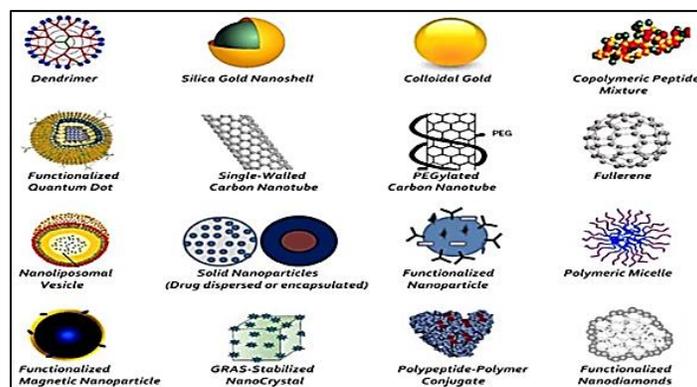


Figure 7: Types of nano system

Nanoparticles contains the unique physicochemical properties, and it might use in the treatments of various chronic diseases or infections in the future. Previously, this nano medicine has showed that metal oxide nano-material have anti-bacterial activities [42]. It has be situated and established that silver nanoparticles, silver ions (NPs Ag), and nanosilver-ions covering complexes have anti-bacterial activity with high ability to deactivate viruses and bacterial growth, and other study reports brief about titanium dioxide nanoparticles (TiO₂ NPs), gold nanoparticles (Au NPs), magnesium oxide nanoparticles (MgO NPs), zinc oxide nanoparticles (ZnO NPs), etc. it have some anti-microbial activities [43,44]. Nanotechnology is permitted the formation of nanoparticles drug delivery systems like microcapsules, liposomes, and micro-emulsion [45]. Size reduction approaches and machineries produce various types of nanostructures that reveal distinctive biological and physicochemical stuffs. These techniques are providing the nanoparticles promising substantial for biomedical applications and thus acquire the significance importance in pharmaceutical sciences. Further developments in this technology, it may cause improving the drug targeting, such as enhancing release, reducing toxicity, bioavailability, improving solubility and provide better formulation chances for drugs. Nanotechnology provides drugs in the range of nanometer size which increases the performance in different types of dosage forms.

Liposomal delivery and nano emulsions:

Liposomes being manufactured synthetically using cholesterol or any other natural phospholipid, which are tiny and spherical in nature. The advantages of liposome over conventional dosage form are their particle size, HLB, entrapment efficiency and their compatibility in the human system. In most of the studies, liposomes showed maximum number of anticipated clinical use of all the nanomedicines in treatment for leishmaniasis in present days. Liposomal preparation of amphotericin B is one of the best examples for the treatment of leishmaniasis (Figure 8) [46].

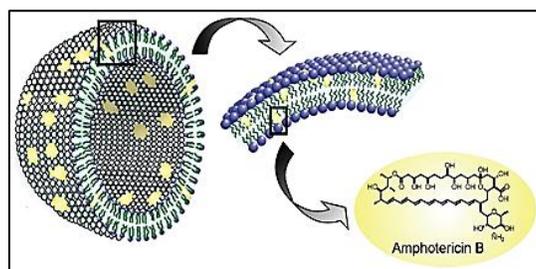


Figure 8: Liposomal preparation of amphotericin b

Perez, analyzed in a study that *in vitro* antileishmanial action of liposomes which have various destortability functions are burdened with a light sensitizer like zinc phthalocyanine (ZnPcAL) [47]. He compared both the liposome systems; one is with soyabean phosphatidylcholine, total polar archaeolipids (TPA's) sodium cholate, and the other with an ultra-deformable character, free of TPA's. He observed that the photoactive liposomes were not damaging ptomastigotes, therefore a less concentration of 0.01mM of zinc phthalocyanine and phospholipids corresponding to 7.6 mM which were exposed to very low energy radiations that eliminated *L. braziliensis* from J774 macrophages without reducing the feasibility of the host cells, HaCaT keratinocytes and dendritic cells that are derived from bone marrow (Figure 9). Interestingly, it was found that the liposomes containing TPA's were only

absorbed by macrophages which successively led to increase in the delivery intracellularly by 2.5 folds in comparison with the ultra-deformable liposomes (UDL).

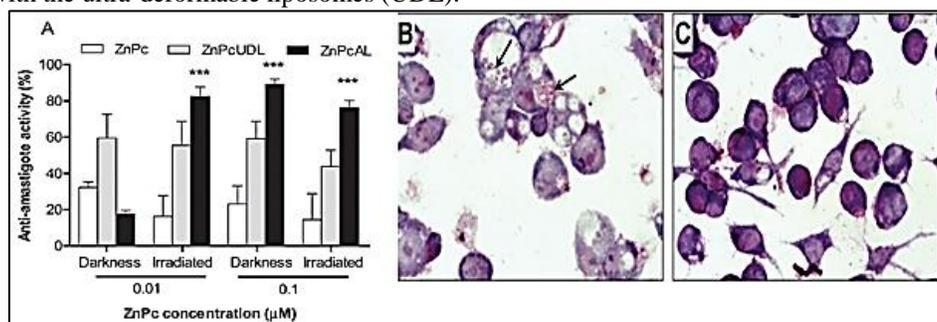


Figure 9: A) The efficacy of anti-amastigote in free and liposomal formulation; B) optical microscopy of infected cells with free zinc phthalocyanine; C) optical microscopy of infected cell with UDL

Kansal [48] found that the preparation of nanocapsules (NC's) by using layer by layer method nanoemulsion core which is loaded with doxorubicin which is coated with the phosphatidylserine (PS) to increase the uptake by the cells. The potency of nanocapsules of doxorubicin (NCS-DOX) and doxorubicin nanocapsules coated with phosphatidylserine (PS-NCS-DOX) to target *L. donovani*. Cellular uptake by J774A.1 macrophages, intracellular localization, organ distribution studies and *in vivo* pharmacokinetics were studied. When tested for the anti-leishmanial activity of NC's-DOX and PS-NC's-DOX, PS-NC's-DOX showed an enhanced uptake by J774A.1 macrophages cell lines when compared to the NC's-DOX especially in organs such as liver and spleen. It was also observed that the anti-leishmanial activity with PS-NC's-DOX showed a parasitic inhibition of 85.23% whereas the NC's-DOX and the DOX showed the parasitic inhibition of 72.88% and 42.85% respectively (Figure 10) [49].

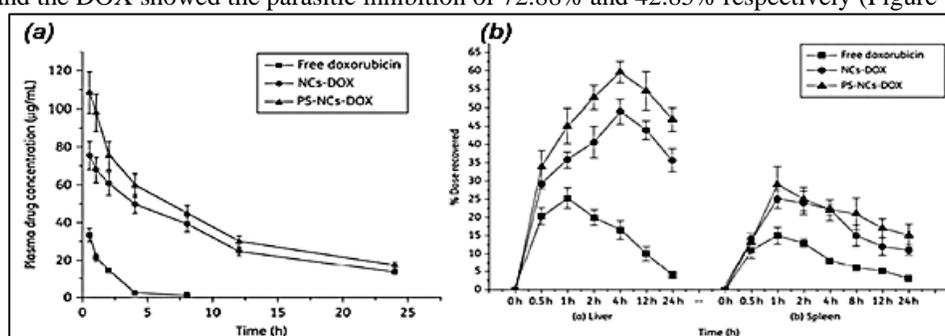


Figure 10: (a) Plasma drug time profile of doxorubicin in wistar rats; (b) splenic uptake of the drug from different formulations of doxorubicin

Nanosphere of PLGA containing Amphotericin B (AmB-PLGA-NS) was prepared by Costa Lima et al. which had a significant anti-leishmanial activity [50]. A single shot injection of PLGA-NS, free AmB and AmB-PLGA-NS to the mice infected with promastigotes. The results revealed that the AmB-PLGA-NS was efficacious and showed preferential accumulation in the organs. In addition to these advantages it was observed that the immune system was modulated which improved the treatment effectiveness. The cytotoxicity test was performed using the cell mediated cytotoxicity kit which showed that the efficacy of AmB-PLGA-NS was due to the effect of CD8 t-lymphocytes, which was demonstrated by the researcher. Another study carried out by Roy et al. have prepared nanoparticle using a diterpenoid lactone andrographolide which is extracted from the leaves of *Andrographis paniculata* that showed a greater anti-leishmanial activity with a low toxicity to the cells. The nanoparticles were prepared by using the drug:polymer ratio of 50:50 where the polymer used was poly(DL-lactide-co-glycolic acid) which is stabilized using PVA [51]. Macrophages in Albino mice for Anti-leishmanial activity were found to be important for nanoparticle preparation with 4% PVA in about one-fourth of the dosage of pure compound AG. Later on the researchers concluded that this compound provides chemotherapy at a comparatively low cost by an alternative mechanism. A study was conducted to appreciate the treatment effects of *L. major* infected mice with ferroportin (Fpn) nanoparticles showed resistance to the *L. major* but were susceptible to visceral infections, anemia and finally death. To express Fpn-EGFP protein, fragment encoding Fpn amplified and sub-cloned to a GFP expression vector. For oral administration for the *L. major* infected BALB/c mice, this vector was further loaded into alginate/chitosan polymeric NPs. In treated and untreated mice after administration, the treated mice showed higher hematocrit,

decrease in footpad size, iron level and parasite load measurements. In treated mice, decreased levels of IL4 and IL10 and increased ratios of IFNG/IL4 or IFNG/IL10 were observed. On treatment of BALB/c mice with alginate/chitosan NPs loaded with Fpn-encoding exhibited significant reduction of *L. major* infection, improved anemia and immune system in the animal model used for leishmaniasis was observed [52]. To overcome the stability issues and bioavailability barrier, Artemisinin was encapsulated into PLGA NPs were prepared, characterized and statistically optimized using Box Behnken design. These NPs were spherical in shape; exhibited 221 ± 14 nm and sustained release kinetics were observed. Results shows significant reduction in amastigotes/macrophage and percentage infected macrophages *ex vivo*; when compared to that of free artemisinin and were non-toxic in contrast to free compound [53]. ROS is known for exhibiting anti-microbial activity. From the reports, TiAg-NPs can act via generation of ROS which are prepared and tested for anti-leishmanial activity against *L. tropica* and *L. infantum* species. Reports confirmed that reduced viability values of promastigotes for both species; up to 3 fold and 10 fold respectively in dark and about 20 folds for both in presence of light. TiAg-NPs in dark were able to inhibit survival by 2-2.5 folds, whereas TiAg-NPS in visible light inhibited 4-4.5 folds of both parasites. So mixture of TiAg-NPs with light serves as a assuring alternative against VL treatment [54]. Cury et al. designed liposomal systems of cinnamic acid derivatives and tested them against cultures containing mixture of *Leishmania amazonensis* stain and DMSO (dimethylsulphoxide) solution. They reported noticeable interactions of derivatives with lipid bilayer during evaluation and accepting system for eradicating of leishmaniasis was concluded [55]. The treatment for leishmaniasis is primarily based on chemotherapy, but these drugs have low potency and safety at low costs. Dinitroanilines are novel moieties with proven *in vivo* anti-leishmanial activity, but it is less soluble in water and accumulates less at infected sites are major problems in formulation development [56]. Considering this, Lopes et al. developed SLNs and liposomes of oryzalin, and related there *in vitro* and *in vivo* clinical potential. Oryzalin addition in this lipoidal system resulted in better bio distribution profile with less haemolytic activity and cytotoxicity with respect to free form. In addition, results of therapeutic potential assessed using VL murine model disclosed decreased parasitic burden in spleen and liver for both nanoformulations (including glucantime); indicating their major application in VL. In other way, researchers also developed liposomal system of hemi synthetic trifluralin derivatives to enhance antileishmanial activity. The *in vitro* and *in vivo* evaluation was done using promastigotes of *L. infantum* and intracellular amastigotes on a zoonotic VL murine model respectively. The system showed decreased cytotoxicity and hemolytic activity with short amastigote loads in the mice spleen. Carvalheiro et al. concluded that Nano therapeutic approach with addition of new synthetic molecules would be better plan for effective management of VL [57].

Natural products:

The pharmaceutical industry shows interest to develop a product from plant and mineral sources it have been create new delivery system based on the traditional medicine to treat different disease like leishmaniasis. In fact 80% of developing countries adapt traditional medicine because currently, this system creates good impact on the people. Worldwide many laboratories are described as the active product extract from different plants. De Queiroz [58] examined the anti-leishmanial activity from various species of plants, these plant extract used in traditional medicine for patients with CL (*L. amazonensis*). The extracts of *Pfaffiaglomerata* (Spreng) Pedersen, *Aloevera L*, *Hyptis pectinata L Poit*, *Rutagraveolens L*, and *Chenopodium ambrosioides L* showed potential activity against extracellular forms of Leishmaniasis. The recent study of thymol derivatives were they are tested in mice and founded that thymol derivatives show good activity compare to eugenol derivatives, the conclusion was benzoyl-thymolis the great inhibitor with minimum toxicity, and further development of this compounds are used to treat leishmaniasis [59]. One of the *in vitro* and *in vivo* studies reported as a plant leaves of *Artemisia annua* it produce essential oil; this oil shows better bacterial against *L. donovani* [60]. Kaur [61] conducted study on herbal drugs derived from *Withaniasomnifera* and *Asparagus racemosus* as a whole plant extract used to treat infected *L. dovovani* BALB/c mice. This study mainly dealing with the both mixtures and the conclusion was, these mixtures have great protective mechanism against parasites by developing the less toxic Th1-type of immune responses. Diagnose the current immunogens as vaccine in a murine model of Visceral Leishmaniasis, these are epitope-based immunogens system, it contain the phage-fused peptides mirroring the *L. infantum* antigens, the study was conducted antigen selected and affinity of antibodies from asymptomatic and symptomatic VL dogs sera [62]. Two phage clones stating target mimotopes provided safety in mice against *L. infantum* matched to a Th1-type immune response. Before, it was reported that liposomes linked with AmB were used for Leishmaniasis treatment [63]. Moreover, study conducted and found that this liposomal drug delivery have potential adjuvants for various antigens and effective transporters for proteins and peptides, enhancing that immunogenicity. This carrier system containing proteins of glycosylphosphatidylinositol-anchored these proteins are decreasing the growth of *L. amazonensis* promastigotes and it creates harmless immunity in mice [64].

Nanotube

Prajapati *et al.* created a nanotube based drug delivery system it contain multi-wall nanotubes which used overcome the drug induced toxicity of anti-leishmanial drugs. This system developed by the functionalized carbon nanotubes (f-CNTs) are bonded Amphotericin B drug and its yields Amphotericin B-f-CNTs. The drug delivery system improved the drug potency for prevent the development of *L. donovani* species growth. Amphotericin B-f-CNT was detected to be fourteen folds high effective than Amphotericin B for preventing the culture growth of amastigotes. Furthermore, *in vitro* and *in vivo* laboratory study shows reduce toxicity to liver and kidney in mice was noticed. Interestingly, the more suppression percentage growth of parasites in the mice spleen with Amphotericin B-f-CNT (89.8%) was attained compared to Amphotericin B (68.9%) [65].

Vaccine

Vaccines are the biological preparation, which provides active acquired immunity to a specific disease. For leishmaniasis no vaccines are available to prevent the infection, cost of development and political involvements are the reasons of unavailable vaccine in a market. But currently vaccines are used prevent the human cutaneous leishmaniasis have trained for periods. The vaccines are formulated to overcome the parasite resistance of anti-leishmanial drugs and this vaccination as a substitute to chemotherapy of anti-leishmanial drugs. The various studies of vaccines are conducted to reach certain applications and efficacies product. Thoughtful vaccination is of *virulent* species from the excretion of avigorouslaceration is the ancient exercise [66]. The various types' vaccines are classified based on their preparation such as live attenuated *leishmani* parasite (first generation), recombinant *leishmania* proteins (second generation), and immunomodulators. The different novel drug delivery systems are explored and exhibited promising results for potential vaccine candidature. These drug delivery strategies have been evaluated for its potential stimulation of Th1 type immune response against the severe disease. In Russia the first vaccine was developed by the culture of Promastigotes *L. major* to encourage treatment against leishmaniasis [67]. After several trials the Israeli scientists developed the standard for inoculums of culture promastigotes and this process known as 'leishmanization' [68]. This process still used in many countries, such as notably Uzbekistan [69]. But, the vaccination has many basic difficulties to prevent the extensive use to treat CL, with difficulty in standard developing the virulence of the vaccine and intermittent is more, determined wounds show from inoculum [70]. The vaccination is a basic antigen preparation of several species of *Leishmania* gained from promastigote forms, the various clinical trials shows that with or without using bacillus of Calmette and Guerin (BCG) as adjuvant, had been verified against VL and CL. For determining possible vaccine candidates, phage display technology was employed to screen new immunogens as potential candidates and analyzed against VL in murine model for developing cost-effective prophylactic strategy. Epitope based-immunogens with an ability to mimic *L. infantum* antigens were identified as a possible candidate based upon their affinity to anti-bodies from asymptomatic and symptomatic VL dog's sera.

First-generation vaccines:

The first- generation of vaccine is the live attenuated vaccine it is prepared by killed bacterial species. In Brazil several *in vitro* and *in vivo* studies was conducted mayrink, in 1980 the first vaccine was showed 53% of efficacy by cellular immunity determined in leishmanian skin test. The proper understanding of diagnosis of disease is the main reason for effectiveness of the vaccine.

Second-generation vaccine development:

The second generation vaccine is developed by recombinant technology, in this technology used to formulate various types of *leishmani* proteins. The following characteristics are helps to prevent against *Leishmania* species, such as (i) vaccine must provide safe and effectiveness; (ii) a less number of immunizations, it must be a long-term protection against human pathogens; (iii) the vaccine should be economic; (iv) the product must be free from contamination; and (v) it must be shows results in both preventing and treating leishmanial infection. Other features like single dose vaccine, and a needle-free it also listed. Vaccine formulators should focus to meet both regulatory and scientific standards.

Currently Various Types of Drug Delivery Devices

The currently various types of drug delivery devices are formulated like nanodisks, and nanospheres with the use of this system the amphotericin B drug is loaded and *in vitro* and *in vivo* laboratory studies tested on experimental BALB/c infected mice administered with intraperitoneal route. Recently Veerareddy established coated-mannose and uncoated delivery system of lipid nanospheres of Amphotericin B. Two types of formulation are prepared and

tested in different groups of mice. The formulations of coated and uncoated lipid nanospheres are administered in *L. donovani* infected BALB/c separate group mice at a minimal effective dose are provided (5 mg/kg body weight), and same dose of uncoated Amphotericin B lipid nanospheres administered with fungizomes in separate mice as control group. These formulations of Amphotericin B lipid nanosphere conclude that enhanced the potency of the drug to interact with ergosterol. Nanospheres formulation did not result any kind of development in the Amphotericin B activity against the resistant organism and it is categorized in the lack of ergosterol.

CONCLUSION

The diversity and harshness of the infection, increasing resistance of antileishmanial drugs and unavailability of suitable marketed vaccination for the leishmanial infection has alarmed the need for developing novel therapeutic strategies to modulate and counter-act this severe parasitic burden. Consequently these have led to an increase in research for suitable drug delivery systems for administration of currently available anti-leishmanial drugs to overcome their resistance and toxic side effects, as well as to increase their efficacy. High persistence of the infection further necessitates more focused research for finding potent and effective alternatives in therapy.

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