ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 10, Issue, 01, pp.64103-64113, January, 2018

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

REVIEW ARTICLE

VESICULAR DRUG DELIVERY AGAINST LEISHMANIASIS IN ANIMALS: A CRITICAL EVALUATION

***Ardhendu Kumar Mandal**

Infectious Diseases and Immunology Division, CSIR-Indian Institute of Chemical Biology, India

ARTICLE INFO

Article History:
Received 09th October, 2017
Received in revised form
15th November, 2017
Accepted 21st December, 2017
Published online 19th January, 2018

Key words:
Leishmaniasis; Macrophage;
Host-parasite interaction;
Vesicular drug delivery.

ABSTRACT

In spite of rapid advances in the development of pharmaceutical medicines, the targeting of drugs to host macrophage, the key cell responsible for body's defense as well as immune response against invading *Leishmania* parasite, the causative agent for kala-azar or leishmaniasis, still remains unexplored. Perturbation of macrophage surface by parasites leads to activate oxidative burst and signal transduction for the interaction of host-parasite i.e. macrophage with *Leishmania* parasite. Amastigotes residing within macrophage phagolysomal compartment are the pivotal target for anti-leishmanial treatment. In general, free drugs cannot overpower main configurational barriers. Moreover, restrictions on ongoing therapy regarding toxicity, efficiency, cost, and duration of treatment may influence in enhanced parasitic resistance. Therefore, vesicular (liposomal and nanoparticulated) drug deliveries have emerged as alternative conventional approach for having not only their improved bioavailability, non-immunogenecity, reduced toxicity and higher carrier capacity but also for promoting encapsulated drug release in parasite-loaded intracellular macrophage cell in a sustained manner.

Citation: Ardhendu Kumar Mandal, 2018. "Vesicular drug delivery against leishmaniasis in animals: A critical Evaluation", *International Journal of Current Research*, 10, (01), 64103-64113.

INTRODUCTION

Parasitic protozoa belonged to the genus *Leishmania* causes a wide spectrum of diseases collectively called leishmaniasis. Kala-azar or visceral leishmaniasis, caused by *L. infantum, L. donovani*, and *L. chagasi,* is potentially fatal to human beings if remains untreated exhibiting the rate of mortality over 95%. The life cycle of *Leishmania* is made up of extracellular promastogote, cultivated in the midgut of sandfly vector and intracellular amastigote nurtured within host macrophages while promastigotes are transmitted through biting of sandfly vectors from one host to another. The macrophage has important roles in defending host against invading parasites and injury, in immune responses and in endocytosis related with receptor-ligand interactions. Thus the macrophages take part a crucial role by phagocytosing the parasites within themselves to kill and by producing immune-regulatory and effector -cells of parasitized animals. Macrophages can tie parasites through specific or non specific receptors to internalize them by phagocytosis into phagolysosome compartment through phagosome formation for parasitic degradation by lysozyme. The interactions between parasite-surface and the host macrophage within phagolysosome during parasitism is vital where parasites take out nutrients for their survival and interrupt the host parasiticidal actions, the target site for leishmanial chemotherapy (Chakraborty and Basu, 1997). Drug resistance and toxicity are main obstructions in the leishmaniasis-therapy with the most potent drugs such as pentavalent antimonial, pentamidine and amphotericine B (Chakravarty and Sundar, 2010; Pal *et al*., 2001; Bhattacharyya *et al*., 2001). Biomedical science has elaborated the necessity to regulate, control and target the drug-release in the body to the specific site of interest. Accordingly, the primary requirement has been emphasised on providing less frequent administration of drug with a sustained release in the systemic circulation or at the specific target site furthering reduced toxicity. Among various strategies for site-specific drug targeting, vesicular carriers were found to be suitable for their structure function diversity in in vitro and in vivo applications. By using mannosyl-fucosyl receptors on the reticuloendothelial macrophage cells -surface, mannose grafted liposomes were monitored to be efficient in the antileishmanial drugs - delivery to these macrophage cells with a remarkable parasitic load -decrement in the spleen of animals (Banerjee *et al*., 1996). Furthermore, consideration of available few chemotherapeutic agents and inventive antileishmanial medicines, paralleled with the investigation for less toxic and more effective antileishmanial drugs together with vaccine development, has forced to draw plans of steady nanotechnology derived drug delivery system to be the

Corresponding author:* **Ardhendu Kumar Mandal
Infectious Diseases and Immunology Division, CSIR-Indian Institute of Chemical Biology, India

principal strategy in combating leishmaniasis. The usage of colloidal vehicle encapsulated with lead drug components, is a potent therapeutic perspective for the physiological uptake of systemic administered mononuclear phagocyte -system i.e. nanosystem leading to higher drug concentrations in the intracellular parasitized macrophages with a protection of in vivo drug degradation (Sana *et al*., 2017; Pei and Yeo, 2016).

Interaction between macrophage and *leishmania*

***Leishmania* parasite entry**

The host-parasite interactions between *leishmania* parasites and various macrophage inhabitants may be happened by (i) chemotaxis, (ii) interlinking at the macrophage surface through receptor-ligand attachment, (iii) different aspects of parasite entry to macrophages, and (iv) final destiny of *Leishmania* in intercellular compartments.

Parasite infection has been recognized on the macrophage surface of parasite ligand with their corresponding receptor (Chang and Fong, 1988). Upon entering the host, the chemotactic substances for *Leishmania* parasites may be released (Bray, 1983). *Leishmania* may bind themselves to the macrophage cells before entrance. Macrophage cells have binding sites on their plasma membrane for about forty ligands associated with phagocytosis following adhesion. The foremost vital receptors in phagocytosis and adhesion refer (i) the mannose-fucose receptor (CR3), (ii) the fibronectin receptor (FcγRI, FcγRII, CR1), and (iii) the updated glycosylated brand receptor. Many parasite ligands are also involved during interaction with macrophages (Bray, 1983).

Two typed attachments have been monitored for *L. donovani* promastigotes relating the macrophage surface: binding in the absence and / or presence of serum opsonins. In absence of sera, the attachment of hamster macrophages to *L. donovani* promastigotes relates saturation kinetics depending on temperature and calcium requirement following the mannose / N-acetyl glucosamine receptor-mediated macrophage -persuaded endocytosis. The enhancement of the receptor sites through modulation of macrophage membrane by cholesterol enrichment, increases the attachment of macrophage surfaces to the promastigotes (Mukherjee *et al*., 1988) suggesting the binding of promastigotes to macrophage lectin-like receptors through glycolipid or glycoprotein ligands -containing N-acetyl glucosamine, glucose, mannose, lipophosphoglycan (LPG) or sialic acid remnants. Moreover, the binding of macrophages to *Leishmania* promastigotes could be caused to CR3 identifying C3bi on the *Leishmania* parasite surface (Blackwell *et al*., 1985). However, this binding can be partly restricted by mannose-rich mannan. According to several studies, the major surface glycoprotein, gp63 may also attach the macrophage surface in a receptor dependent way. Serum dependent attachments of *Leishmania* promastigotes to host macrophages are occurred by opsonins-adsorption such as antibodies and complement by the promastigotes and macrophage-C3b receptors while Fc receptors are utilized for the binding of parasite opsonised with specified antisera. In the *Leishmania*-macrophage interaction, parasites are interiorized through coated pits at a high rate via receptor-mediated endocytosis accompanied by the fusions of the parasitophorus vacuoles with secondary lysosomes forming phagolysosome (Blackwell *et al*., 1985) while amastigotes of *Leishmania* parasite colonize inside phagolysosomal compartment of the macrophage and induce the enlargement of the compartment to form huge parasitophorous vacuoles (Peters *et al*., 1997). The secreted amastigote product, proteophosphoglycan creates peritoneal macrophages - vacuolization in vitro and may influence to expand the infected phagolysosomal compartment in vivo (Peters *et al*., 1997).

Eventual sequences during and after interiorization of *Leishmania* parasite

Respiratory burst

The triggered respiratory burst as well as the oxygen metabolites -production occur at the macrophage surface during internalization of *Leishmania* parasite. Perturbation of the macrophage membrane by the *Leishmania* parasite may induce to trigger the membrane-bound NADPH oxidase to catalyze the molecular O_2 reduction to O_2^- enhancing the oxidative activity. The activated macrophages can kill the parasites by two ways:

Oxygen Dependent Mechanism

The oxidative burst links biochemical serial events while NADPH generated in the hexose monophosphate shunt is utilized as substrate given below:

Oxygen consumption and the production of O_2^-, H_2O_2:

Oxidase
$2O_2+NADPH \rightarrow 2O_2^- +NADP+H^+$ (At the phagocyte-surface)

SOD
$2O_2^-+2H^+ \rightarrow O_2+H_2O_2$

$2H_2O_2+catalase \rightarrow O_2+2H_2O$

Oxygen independent mechanism

This mechanism relates nitrogen oxides production which mediates intracellular parasite destruction by lymphokine-triggered macrophages (James and Nacy, 1993). The reactions occurred in this mechanism involve the NO_3^-, NO_2^- and NO - synthesis from L-arginine (James and Hibbs, 1990). Arginosuccinate synthetase and nitric oxide synthase participate in the NO production (Nussler *et al*., 1994) while nitric oxide synthase may be regulated by protein tyrosine kinase (Dong *et al*., 1993). Iron-loss from the crucial target enzymes by the interaction with nitrogen oxides causes metabolic failure of intracellular parasites (Dong *et al*., 1993). The toxic O_2 metabolites could mediate damages to *Leishmania* parasites due to inactivation of iron-supported superoxide dismutase (James and Nacy, 1993). The toxic activities of NO are inter related with toxic O_2 intermediates and also suggest the elimination of intracellular *Leishmania* amastigotes by IFN-γ triggered macrophages related to NO production (Lin and Chadee, 1992; Liew *et al*., 1990).

Survival criteria of *leishmania* parasite within phagolysosome

Leishmania amastigotes survive within the acidic intralysosomal macrophage compartment. The fate of intracellular *Leishmania* parasites and the ultimate disease

development depend on the different *Leishmania* species and the host immune response. The parasite shows different strategies to enable evasion of host defense system (Bogdan *et al*., 1990).

Parasite resistance to sera contents and phagolysosomal activity

Some infective *Leishmania* species are not capable to activate the alternate complement pathway and resistant partially to sera mediated destruction. Sometimes, surface antigens regulated by development, enable the *Leishmania* pathogen to become resistant to the classical complement system (DaSilva *et al*., 1989). Comparatively few other *Leishmania* amastigotes are resistant to lysis by human sera killing in spite of C3 fixation and attachment while opsonisation of *Leishmania* promastigotes by C3bi allows the entrance of it via host cell CR3-receptor. *Leishmania* parasites -survival within phagolysosome relates oxidative burst inhibition, oxidative metabolites scavenging, lysozymes inactivation and down-regulated O_2- dependent killing (Buchmuller-Rouiller and Mauel, 1987). Amastigotes display high activities of glutathione peroxidase and superoxide dismutase for toxic macrophage intermediates -degradation. Some molecules such as gp63, LPG, nucleotidase, cysteine proteinase and acid phosphatase, existed on *Leishmania* parasite cell –surface, are responsible for its virulency as well as survival (Choudhury and Chang, 1990) while LPG and gp63 function as ligands for CR3 and CR1 macrophage receptors to enable pathogen entry via these receptors triggering little respiratory burst (Wright and Silverstein, 1982). LPG generally restricts oxidative burst, lysosomal β galactosidase activity and can scavenge reactive oxygen species while gp63 inactivates host proteolytic lysozymes protecting pathogen proteins from phagolysosomal decay. *Leishmania* parasites have also the ability to keep up a neutral intracellular pH for survival in spite of acidic pH inside the phagolysosomal compartment (Bogdan *et al*., 1990).

T cell immune response during infection

Analysis of immune responses in mice during *Leishmania* parasite infection has focussed the host susceptibility and resistance persuaded by functionally diverse $CD4^+$ T lymphocytes (Muller *et al*., 1989). Th1, one subset of $CD4^+$ T lymphocytes, produces high quantity of IFNγ, while Th2, the other set, secretes IL4 cytokine, involved to control the disease progression. Host-resistance is accompanied by Th1 cell cytokines such as IL2 and IFNγ, whereas susceptibility by Th2 type cytokine IL4. *Leishmania* parasitized macrophages are effective in arresting the Th1 type immunity such as IFNγ persuaded macrophage triggering by restricting MHC class II antigen expression and IFNγ mediated signalling (Reiner *et al*., 1988) while IFNγ receptor and tyrosine phosphorylation of Janus activated kinases (JAK kinases) are downregulated, and signal transducer and activator of transcription (STAT1) is diminished (Ray *et al*., 2000; Nandan and Reinerb, 1995). IL12 takes part in the Th1 type immune response against *Leishmania* parasite by induction of IFNγ and by prolonging sufficient quantity of memory / effector Th1 cells (Reiner *et al*., 1994; Stobie *et al*., 2000). The selective impairment by *Leishmania* parasites to the production of IL12 by host cells, ensures their survival within host macrophages (Carrera *et al*., 1996). Sand fly saliva also increases parasite evasion of the host immunity by facilitating enhanced IL10 production (Norsworthy *et al*., 2004).

Regulation of receptors

The process of attachment through specific receptors mediated internalization or endocytosis becomes faster when infective strains of *Leishmania* parasites expressing surface LPG and gp63 are endocytosed by host macrophages (Chakraborty *et al*., 1996). Decreased binding for sugar-specific receptors as well as mannose or glucose-bearing liposomes has been monitored for macrophages infected earlier with *Leishmania* parasite in vitro or in vivo, due to receptor down regulation for decrement in the number of receptor sites having no alter for inclination (Dutta *et al*., 1994; Basu *et al*., 1991).

Immuno-effector mechanisms in *leishmania*-macrophage interactions

Presentation and processing of antigens

Host macrophages internalize parasite macromolecules by phagocytosis, which is then transported to phagolysosome for chewing up by the proteolytic enzyme and the relevant escaped part is translocated to the surface associated with major histocompatibility complex (MHC). The T-cell receptor docks on this peptide-MHC complex leading cell triggering. The antigen presentation is believed to be induced by Nramp1 protein in *Leishmania* parasite to regulate macrophage activation through controlling expression of MHC-II molecules. Macrophages transfected with natural or wild type mutant allele of the Nramp1 gene were utilized to investigate MHC class II expression, processing and presenting recombinant antigen protein to $CD4^+$ T-cell hybridoma. Transfected macrophage clone possessing the wild type allele exhibited increased class I molecules up-regulation in response to IFNγ contrasted with macrophage clone having mutant allele, and also showed an increased lipopolysaccharide based capability to present the recombinant *Leishmania* parasite antigen LACK-delta1 to LACK-specific CD4+ T-cells influencing the potential contribution of Nramp1 gene in infection and susceptibility to autoimmune disease (Lang *et al*., 1997). The generation of $CD8^+$ T-cells by specific leishmanial antigen, GP46/M-2 to immunize mice against *Leishmania* parasite has been demonstrated (Kima *et al*., 1997). In addition, $CD8^+$ T-cells also identified macrophages carrying *Leishmania* parasite. Infected macrophages process for MHC I presentation of GP46/M-2 which may be inhibited by the treatments with brefeldin A or specific inhibitors suggesting the process of leishmanial antigen in the macrophage cytosol and its presentation to $CD8^+$ T-cells through the classical MHC I signalling (Kima *et al*., 1997).

Production of cytokines

Leishmania infected macrophage produces a lot of cytokines e.g. TNF-α, IL-1, IL-12, IL-18, etc. It has been observed that IL-18 and TNF-α –releases are higher by polymorphonuclear bone marrow derived cells (PBMC) and monocytes infected by *L. donovani* compared to *L. major* (Iannello *et al*., 2003). There is a report that IL-12 is very crucial for protective immune response against *L. major* (Mattner *et al*., 1996) while in comparison to the pathogenic metacyclic promastigotes, the procyclic forms collected from logarithmic culture-phase exhibited a significant capability to induce IL-10, IL-12 and TNF-α. However, it was monitored that infected macrophages lost their capabilities to generate IL-12 from Th 1 responders in response to LPS or IFN-γ. Furthermore, though the response

of IL-6 was checked partially, the reaction of TNF-α of infected macrophages was noticed to stay unimpaired. Among other cytokines, IL-4 and IL-10 were monitored to inhibit killing of *L. infantum* and *L. major* intracellularly by macrophages with reduced nitric oxide production while IL-10 was observed to be more potent than IL-4 regarding inhibitory activity (Vouldoukis *et al*., 1997).

Role of chemokines

Chemokines, responsive for contraction of chemoattractant cytokines, are familiar to cue particular subtypes of leucocyte to the inflammed sites. They are identified as beta chemokines (C-C), alpha chemokines (C-X-C), lymphoactin and fractalkine (Rossi and Zlotkin, 2000). It is demonstrated that self-healing cutaneous leishmaniasis involves higher monocyte chemoattractant protein -level, MCP-1 for stimulating microbicidal mechanisms of macrophages with infiltration into the lesion (Badolato *et al*., 1996; Moll, 1997). *Leishmania* promastigotes of stationary phase induce speedy and transient transcript expressions of chemokines KC and JF in bone marrow derived macrophages to about 4 to 6 fold rise quickly after ailment and returns to un-induced levels by 4 to 24 h. Chemokines bind with their receptors and function via G-protein coupled receptor(s) (GPCRs) localized on the target cell membranes. The identified chemokine receptors such as CXCR1 to CXCR5 bind to α-chemokines, CCR1 to CCR9 attach to β-chemokines, XCR1 attaches to lymphotactin, and CX3CR1 attaches to fractalkine, and deploy their biological activities in leukocyte recruitments and Th1/Th2 cytokine responses in the leishmanial pathobiology (Roychoudhury and Roy, 2004). There is also proof that chemokines when bind to their receptors, control movements and interactions of antigen presenting cells e.g. T-cells and dendritic cells.

Signalling pathways

Cell surface receptors become activated by external signals and initiate signallings where informations are transmitted from one component to another to produce ultimate effectors. *Leishmania* parasites survive within phagolysosome of host macrophages and the survival is dependent upon the inhibition of signalling pathways by host cells to activate macrophages for generating killing components such as O_2^- and NO against parasites. Activated PKC-mediated signalling events generate O_2^- whereas activated MAPK cell signalling releases proinflammatory cytokine, IL-12 and produces NO. Host resistance against parasites is dependent upon IL-12-mediated Th-1 type protective immune response overpowered by the generation of IFN-γ associated with JAKs, STAT phosphorylations to activate macrophages for killing parasites via NO release while parasites are also capable to impair the productions of IL-12 and IFN-γ by human macrophage and T-cell respectively but the more synthesis of Th-2 cytokines, IL-10 and IL-4 (Rosas *et al*., 2003; McDowell and Sacks, 1999; Reiner *et al*., 1988; Nandan and Reinerb, 1995). It is evidenced that the interactions of CD40 on macrophage and CD401 on T-cell produce IL-10 or IL-12 and activate T-cell to produce IFN-γ by inducing MAPK-mediated signalling while parasites take an attempt to disrupt this interaction as well as signalling for their survival through extracellular signal related kinase (ERK1/2) dependent IL-10 release or p38MAPK dependent IL-12 release signalling. Another adaptor protein myeloid differentiation molecule 88 (MyD88) involves Toll like receptor (TLR) signalling where TLR recognizes *Leishmania* parasite by lipophosphoglycan (LPG), crucial for IL-12 release by macrophage (DeVeer *et al*., 2003). It has been demonstrated that high released Ca^{2+} is accumulated intracellularly in virulent amastigote than in virulent promastigote or avirulent cells of both forms which correlates Ca^{2+}-ATPase and intracellular Ca^{2+} pool contents with Ca^{2+} signals during parasite invasion of macrophages (Lu *et al*., 1997).

Chemotherapy against Leishmaniasis

Parasites, namely, *L. donovani, L. chagasi* and *L. infantum*, are the causative agents of kala-azar (KA) or visceral leishmaniasis (VL), a potent deadly disease found in most tropical countries including the Indian subcontinent if it remains untreated. Organic pentavalent antimonials [Sb(V)] in the forms of sodium stibogluconate such as stibanate, pentostam and soluatibosan or meglumine antimonite such as glucantime are the drugs of first choice for treatment of VL and other forms of leishmaniasis (Berman, 1988). When treatments with these drugs fail, amphotericin B or pentamidine isethionate are used against leishmaniasis as drugs of second choice (Jha, 1983; Mishra *et al*., 1991). Allopurinals have also been used for the treatment of antimony resistant leishmaniasis candidates in Kenya and Bihar, India (Kager *et al*., 1981; Jha, 1983). Drug toxicity as well as its side effects and drug unresponsiveness are the major obstacles in the treatment and control of leishmaniasis. Resistance to antimony chemotherapy in visceral and mucocutaneous leishmaniasis has been recognised as serious clinical health problem (Thakur *et al*., 1998; Arya, 1993; Bryceson *et al*., 1985; Rocha *et al*., 1980; Costa *et al*., 1986; Bryceson *et al*., 1985). It is believed that post kala-azar dermal leishmaniasis (PKDL) is occurred by residual *Leishmania* parasites survived upon chemotherapy and escaped to the skin after clinical cure owing to the resistance to stibanate observed in some patients (Bhattacharyya *et al*., 2001).

It was also noticed that *Leishmania* parasites were cross-resistant to heavy metal ions e.g. Sb^{3+}, Zn^{2+}, As^{3+} and three other drugs such as amphotericin B, colchicines and pentamidine isethionate in addition to Sb(V) resistance (Bhattacharyya *et al*., 2001). To elucidate whether a mechanism of drug efflux system is involved in resistant cells, parasite proteins were analysed by western blot utilizing monoclonal antisera raised against a pellicular membrane drug transporter protein, MRP resulting its higher expression in resistant clones than sensitive one (Bhattacharyya *et al*., 2001). Amplification of P-glycoprotein was also monitored in arsenite resistant cells showing its involvement in drug extrusion as efflux pump from mammalian cells (Pal *et al*., 2001). In addition, the mechanism of Sb(V) resistance in *Leishmania* parasites may be due to intracellular reduction of Sb^{5+} to Sb^{3+} and associate efflux of Sb^{3+}-trypanothione conjugate led by the As-thiol pump (Bhattacharyya *et al*., 2001).

Antileishmanial Delivery Systems

In spite of speedy development in pharmaceutical and medicinal technologies, the delivery of drugs to phagocytes in macrophage-related leishmaniasis persists to be resolved. The use of vesicular drug delivery systems (VDDSs) may stand as a complementary strategy to develop newer treatments as well as combinatorial therapies for leishmaniasis to deliver high drug concentration into the intracellular parasite-loaded phagolysosome or parasitophorous vacuole. The attractive goal

of this type of drug delivery is to pursue maximum amount targeting of small quantity of encapsulated drug compared to high amount of free drug administration to macrophages at a sustained manner avoiding drug-toxicity as side effects. Receptor mediated vesicular drug delivery to parasite infected macrophages was observed to be more potent in comparison to simple vesicular drug delivery in targeting antileishmanial drug to phagocytic cell. This type of delivery system not only overcomes poor solubility of drugs but also protects the active lead compound from decadence in systemic fluids. Moreover, it has given emphasized on the development of vesicular oral formulation to improve patient compliance with this delivery system to overcome also drug resistance with combination therapy. The recent report on the investigation in anti-visceral leishmaniasis technology with special reference to the use of liposomes, nanocapsules, metals, non metals and carbon nanoparticles -encapsulated with drugs used against leishmaniasis has been focussed. Here, the most challenging aspect is the oral drug administration which becomes limited due to its lower absorption by passive diffusion through the intestinal cell membranes based on drug's molecular weight greater than 500Da when efficiency decreases (Italia *et al*., 2009). Moreover, drugs become degraded in the acidic pH and bacterial existence of the gastrointestinal tract supported by the extensive P-gp efflux from the enterocytes. To overcome this problem of drug bioavailability, oral formulations of liposomes and nanoparticles –encapsulated drugs were optimized and applied against visceral leishmaniasis (Torrado *et al*., 2013). Currently, few other herbal compounds encapsulated in liposomal or nanoparticulated delivery system have also been investigated in vitro, and in vivo mice or hamsters models to enlighten the efficacy of new drugs in combating visceral leishmaniasis.

Liposomal drug delivery

Liposomes are microscopic spheres made up of lipids usually phospholipids. They spontaneously form when lipids are dispersed in aqueous media. They can be constructed so that they entrap quantities of highly polar and relatively small solutes within the aqueous compartment and lipophobic substances within the lipid bi-layers. Owing to their stability, biocompatibility, easy scaling up and capability to carry different lead compounds as cargo, different formulations of liposomal drugs have been optimized to target drug to specific site of interest against various diseases (Allen and Cullis, 2013). Following the systemic administration, liposomes are accumulated in the reticuloendothelial system (RES) such as spleen, liver, lungs, kidney, lymph nodes and bone marrow, and cleared up by residential macrophages. In accordance with earlier report, the best clinical outcomes have been gained by the liposomal formulation AmBisome than lipid complex formulations of AmpB regarding efficacy in in vivo evaluations (Larabi *et al*., 2003). Recently, cholesterol has been substituted by ergosterol containing 50% total lipids molarity in liposome formulations as cholesterol enhances the growth of leishmania promastigotes and has been named as Kalsome^{TM}10 whose efficacy was found to be very potent in apoptotic cell death in *Leishmania* parasites (Mishra *et al*., 2013; Asad *et al*., 2015; Shadab *et al*., 2017). A lot of anti-leishmanial antimonials have been formulated in liposomes and their biological efficacies have been evaluated (New *et al*., 1978). Furthermore, liposomes have been modified with anionic and neutral surface charges to test their efficacies (Carter *et al*., 1989), but only cationic phosphatidyl choline-stearylamine liposomes have shown their effective supremacy against ailment with sodium stibogluconate-resistant *Leishmania* pathogens in mice with the enhancement of charged phospholipid binding to macrophages (Roychoudhury *et al*., 2011). Several drugs such as doxil, meglumine antimonite, camptothecin and doxorubicin have been optimized in liposome formulations with polyethylene glycol (PEG) coating to increase liposome stability and enhance blood circulation half-life. Their efficacies have been evaluated as more effective than currently available drugs to decrease the resistance of *Leishmania* parasites (Immordino *et al*., 2006; Azevedo *et al*., 2014; Proulx *et al*., 2001; Keighobadi *et al*., 2015).

Nanocapsulated Drug Delivery

Colloidal polymeric nanocapsule such as FDA-approved poly (lactide-co-glycolide) (PLGA) has taken attention as drug delivery carrier against leishmaniasis not only for its small size, shell-rigidity, non-immunogenecity and biodegradability properties but also for capability of a affordable sustained release of the encapsulated drug to infected cells or tissues (Asthana *et al*., 2013; American Pharmaceutical Review). Nanocapsule's modulation can be carried out by its coating of ligands such as chitosan, poly ethylene glycol to enhance circulation time and for getting maximum uptake to effected cells. Several reports have demonstrated that the efficiency of PLGA nanocapsule-coated AmpB was found to be higher than that of free drug or AmBisome against *Leishmania* organism with less cytotoxicity and hemotoxicity (Van De Ven *et al*., 2012). The AmpB-PLGA nanocapsule carrying d-α-tocopheryl poly-ethylene glycol 1000 succinate (TPGS) may be used against drug resistant *Leishmania* parasites while TPGS may act as P-gp efflux inhibitor and stabilizer to improve their absorption and bioavailability (Zhang *et al*., 2012; Italia *et al*., 2012). Miltefosine (MF) loaded PLGA-PEG nanocapsule also displayed higher efficacy and bioavailability compared to free drug as antileishmanial activity (Kumar *et al*., 2016). Lipid-polymer hybrid nanoparticles (LPNPs) containing AmpB with the anionic core made up of PLGA polymer and TPGS surfactant, and the shell composed of cationic stearylamine lipid, have been applied against leishmaniasis while parasite growth inhibition was higher compared to the carrier without stearylamine or the AmBisome formulation, possibly due to higher uptake into the macrophages and RES organs (Asthana *et al*., 2015). In this regard, the positive charge of LPNPs may attach to negatively charged sialic acid receptors on the macrophage surface causing adsorption-mediated endocytosis.

Mannose-coated PLGA nanoparticles containing AmpB and PEG, resulted more efficient parasites-inhibition compared to both the one without PEG and the free AmpB as an active targeting approach (Nahar and Jain, 2009).

Furthermore, the treatment with mannan-grafted PLGA-AmpB nanoparticles reduced the visceral organs parasite load significantly probably owing to enhanced production of cytokines such as interferon gamma and nitric oxide which are responsible for the organism's defense in combating parasite infections (Barros *et al*., 2015). PLGA nanoparticles containing AmpB and coated with glycoprotein lactoferrin (Lef) showed higher in vivo anti-leishmanial activity efficiency compared to non-targeted and commercial formulations as the N-glycosylation sites of Lcf-glycoprotein are identified by mannose receptors located on monocyte / macrophage and dendritic cells surfaces (Asthana *et al*., 2015;

Silva *et al*., 2012). PEG-PLGA-AmpB nanoparticles adorned with anti-CD14 antibody revealed higher parasite inhibition efficiency compared to free drug as CD14 exists on macrophages, neutrophils and dendritic cells surfaces (Kumar *et al*., 2015).

Inorganic compounds as drug delivery systems

Despite the growing pharmaceutical interest of the majority of organic biodegradable polymer nanoparticles, inorganic compounds such as metallic nanoparticles, carbon-based nanostructure and hydroxyapatite nanostructure display several interesting characteristics with diagnostic and therapeutic functionalities as potent delivery system against infectious agents as well as diseases (Ali-Boucetta and Kostarelos, 2013; Baeza *et al*., 2015). Nanoparticulated metals (Mandal, 2017; Mandal, 2017; Mandal, 2017) might be of interest as microbicidal agents for their capability to produce ROS while silver nanoparticles (Allahverdiyev *et al*., 2011) with or without UV light were monitored to inhibit *Leishmania* parasite in vitro and in vivo by damaging their membrane. Silver oxide nanoparticles with or without UV and infrared irradiation were found to be the highest antileishmanial activity rather than the gradual decrease in activities of gold oxide, titanium dioxide, zinc oxide, magnesium oxide and selenium oxide nanoparticles (Jebali and Kazemi, 2013).

Biocompatible nanoparticles doped with different concentrations of copper or silver showed in vitro efficacies against *Leishmania* parasites by their killing through the production of ROS especially when activated by daylight irradiation (Nadhman *et al*., 2015; Nadhman *et al*., 2014). Recently, AmpB adsorbed spherical silver nanoparticles were observed to show more potent in vitro antileishmanial activity by the production of ROS from the released silver ions and augmented upon visible light irradiation (Ahmad *et al*., 2016).

Carbon compounds, the other inorganic nanomaterials, have also taken interest as antileishmanial device owing to their excellent optical, mechanical and thermal properties where drugs are linked covalently to carbon nanotubes or absorbed strongly by their surface π-π stacking. The intraperitoneal administration of AmpB adsorbed multi-walled carbon nanotubes showed higher percentage suppression of the spleen-parasites than with free drug probably due to the covalent link through an ether bond (Prajapati *et al*., 2011; Prajapati *et al*., 2012). Similar to the inorganic contents of calcified tissues, nanosized calcium phosphate substances, due to their effective dimensions, have shown their better bioactivity and biocompatibility compared to conventional materials. Moreover, calcium phosphate is not only a low-cost drug cargo, chemically stable inorganic minerals but also degraded nontoxic product when metabolized (Kester *et al*., 2008; Chen *et al*., 2012). The in vivo anti-leishmanial efficacy of AmpB-loaded calcium phosphate nanoparticles were assessed stronger compared to marketed formulations or free drug suspension due to higher targeted macrophages-interiorization through clathrin receptor -mediated phagocytosis and increment of Th1-mediated immune-response by nanoparticles with preferential uptake in a sustained manner to macrophage-affluent organs such as spleen and liver (Chaurasia *et al*., 2016). The in vitro activity of pentavalent antimonials-loaded calcium phosphate nanoparticles showed the drug internalization and calcium-elevation into the parasite-infected cells resulting parasite apoptosis (Alvarenga *et al*., 2015).

Future Perspectives

The necessity for other treatments, particularly, for the reduction of the clinical treatment period, guide to screen potent metals and natural products for therapeutic use in leishmaniasis along with currently available potential medicines, organic compounds and unexplored active molecules in medicinal plants (Allahverdiyev *et al*., 2011; Jebali and Kazemi, 2013; Nadhman *et al*., 2015; Nadhman *et al*., 2014; Le Pape, 2008; Mishra *et al*., 2009; Espuelas *et al*., 2012; Pena *et al*., 2015; Castillo-Garit *et al*., 2012; Tariq *et al*., 2016; Polonio and Efferth, 2008; Nagle *et al*., 2014; Sangshetti *et al*., 2015). Several active compounds derived from various chemicals such as napthoquinones, indolyl quinoline analogs, doxorubicin, antimicrobial peptides, disulfiram and OIPC with or without vesicular encapsulation showed antileishmanial activity (Silva *et al*., 2012; Marr *et al*., 2012; Sundar and Chakravarty, 2015; Peniche *et al*., 2015; Fortin *et al*., 2012; Hernandez *et al*., 2014; Fortin *et al*., 2016). Natural compounds such as andrographalide, bacopasaponin C, harmine, MT81, oleanolic acid, bisnaphthalimidopropyl derivatives, artemisinin and curcumin in vesicular forms have been evaluated as potent anti-leishmanial agents (Roy *et al*., 2010; Mondal *et al*., 2013; Sinha *et al*., 2000; Sinha *et al*., 2002; Lala *et al*., 2004; Mitra *et al*., 2005; Ghosh *et al*., 2016; Tavares *et al*., 2012; Costa Lima *et al*., 2012; Yang and Liew, 1993; Want *et al*., 2014; Fouladvand *et al*., 2013; Tiwari *et al*., 2017). Furthermore, betulin loaded carbon nanotube has been utilized recently as leishmanicidal agent to overcome drug resistance by down-regulating the activity of P-gp efflux, however, needed further toxicological evaluation (Saudagar and Dubey, 2014).

Conclusions

The treatment for infectious diseases suffers from drug resistance, low drug bioavailability and solubility, high drug toxicity, low drug potency, non-selectivity and short term drug-action in biological system. To overcome these limitations, several potent drugs have been encapsulated in vesicular system with or without ligand specific binding for targeting cells to get maximum biological efficacies which needed further clinical evaluations. On the other hand, metallic nanoparticles have been utilized as delivery system against infectious diseases which require proper surface modification to get maximum potency with vesicular drug encapsulation attached with specific ligands for site specific targeting to get enhanced efficacy which needed further investigations.

REFERENCES

Ahmad, A., Wei, Y., Syed, F., Khan, S., Khan, G.M., Tahir, K., Khan, A.U., Raza, M., Khan, F.U., and Yuan, Q., 2016. Isatis tinctoria mediated synthesis of amphotericin B-bound silver nanoparticles with enhanced photoinduced antileishmanial activity: A novel green approach, *J Photochem Photobiol B*, 161, 17-24.

Ali-Boucetta, H., and Kostarelos, K., 2013. Carbon nanotubes in medicine and biology-therapy and diagnostics, *Adv Drug Deliv Rev*, 65(15), 1897-1898.

Allahverdiyev, A.M., Abamor, E.S., Bagirova, M., Ustundag, C.B., Kaya, C., Kaya, F., and Rafailovich, M., 2011. Antileishmanial effect of silver nanoparticles and their enhanced antiparasitic activity under ultraviolet light, *Int J Nanomed*, 6, 2705-2714.

Allen, T.M., and Cullis, P.R., 2013. Liposomal drug delivery systems: From concept to clinical applications, *Adv Drug Deliv Rev*, 65(1), 36-48.

Alvarenga, B.M., Melo, M.N., Frezard, F., Demicheli, C., Gomes, J.M.M., Silva, J.B.B., Spezialie, N.L., and Corre, J.D.Jr., 2015. Nanoparticle phosphate-based composites as vehicles for antimony delivery to macrophages: Possible use in leishmaniasis, *J Mater Chem B*, 3(48), 9250-9259.

American Pharmaceutical Review. FDA's regulatory science program for generic PLA/PLGA-based drug products. Available from: http://www.americanpharmaceuticalreview.com/Featured-Articles/188841-FDA-s-Regulatory-Science-Program-for-Generic-PLA-PLGA-Based-Drug-Products/.

Arya, S.C., 1993. Engima of therapeutic failure in visceral leishmaniasis, *J Assoc Physicians India*, 3, 155.

Asad, M., Bhattacharya, P., Banerjee, A., and Ali, N., 2015. Therapeutic and immune modulatory activities of short-course treatment of murine visceral leishmaniasis with KALSOME^{TM}10, a new liposomal amphotericin B, *BMC Infect Dis*, 15(1), 188.

Asthana, S., Gupta, P.K., Chaurasia M., Dube, A., and Chourasia, M.K., 2013. Polymeric colloidal particulate systems: Intelligent tools for intracellular targeting of antileishmanial cargos, *Expert Opin Drug Deliv*, 10(12), 1633-1651.

Asthana, S., Gupta, P.K., Jaiswal, A.K., Dube, A., and Chourasia, M.K., 2015. Targeted chemotherapy of visceral leishmaniasis by lactoferrin-appended amphotericin B-loaded nanoreservoir: In vitro and in vivo studies, *Nanomed*, 10(7), 1093-1109.

Asthana, S., Jaiswal, A.K., Gupta, P.K., Dube, A., and Chourasia, M.K., 2015. Th-1 biased immune-modulation and synergistic antileishmanial activity of stable cationic lipid-polymer hybrid nanoparticle: Biodistribution and toxicity assessment of encapsulated amphotericin B, *Eur J Pharm Biopharm*, 89, 62-73.

Azevedo, E.G., Ribeiro, R.R., daSilva, S.M., Ferreira, C.S., de Souza, L.E., Ferreira, A.A., de Oliveira, E., Castro, R.A., Demicheli, C., Rezende, S.A., and Frézard, F., 2014. Mixed formulation of conventional and pegylated liposomes as a novel drug delivery strategy for improved treatment of visceral leishmaniasis, *Expert Opin Drug Deliv*, 11(10), 1551-1560.

Badolato, R., Sacks, D.L., Savoia, D., and Museo, T., 1996. *Leishmania major*: Infection of human monocytes induces expression of IL-8 and MCF, *Exp Parasitol*, 82, 21-26.

Baeza, A., Collila, M., and Vallet-Regi, M., 2015. Advances in mesoporous silica nanoparticle for targeted stimuli-responsive drug delivery, *Expert Opin Drug Deliv*, 12(2), 319-337.

Banerjee, G., Nandi, G., Mahato, S.B., Pakrashi, A., and Basu, M.K., 1996. Drug delivery system: Targeting of pentamidines to specific sites using sugar grafted liposomes, *J Antimicrob Chemother*, 38, 145-150.

Barros, D., Lima, S.A.C., and Cordeiro-Da-Silva, A., 2015. Surface functionalization of polymeric nanospheres modulates macrophage activation: Relevance in leishmaniasis therapy, *Nanomed*, 10(3), 387-403.

Basu, N., Sett, R., and Das, P.K., 1991. Down regulation of mannose receptors on macrophages after infection with *Leishmania donovani*, *Biochem J*, 277, 451-456.

Berman, J.D., 1988. Chemotherapy for leishmaniasis: Biochemical mechanisms, clinical efficacy, and future strategies, *Rev Infec Dis*, 10, 560.

Bhattacharyya, A., Roy, M., Mandal, A., and Duttagupta, S., 2001. Effect of metal ions and other antileishmanial drugs on stibanate-resistant *Leishmania donovani* promastigotes of Indian origin, *Curr Sci*, 81(11), 1470-1473.

Blackwell, J.M., Ezekowitz, R.A., Roberts, M.B., Channon, J.Y., and Sim, R.B., 1985. Macrophage complement and lectin like receptors bind *Leishmania* in the absence of serum, *J Exp Med*, 162, 324-331.

Bogdan, C., Rollinghoff, M., and Solbach, W., 1990. Evasion strategies of Leishmania parasites, *Parasitol Today*, 6, 183-187.

Bray, R.S., 1983. Leishmania Mexicana: Attachment and uptake of promastigotes to and by macrophages in vitro, *J Protozool*, 30, 314-322.

Bryceson, A.D.M., Chulay, J.D., Ho, M., Mugambii, M., Were, J.B., Muigai, R., Chunge, C., Gachihi, G., Neme, J., Anabwani, G., and Bhatt, S.M., 1985. Visceral leishmaniasis unresponsive to antimonial drugs. I. Clinical and immunological studies, *Trans Roy Soc Trop Med Hyg*, 79, 700.

Bryceson, A.D.M., Chulay, J.D., Mugambi, M., Were, J.B., Gachihi, G., Chunge, C.N., Muigai, R., Bhatt, S.M., Ho, M., Spencer, H.C., Meme, J., and Anabwani, G., 1985. Visceral leishmaniasis unresponsive to antimonial drugs. II. Response to high dosage sodium stibogluconate or prolonged treatment with pentamidine, *Trans Roy Soc Trop Med Hyg*, 79, 705.

Buchmuller-Rouiller, Y., and Mauel, J., 1987. Impairment of the oxidative metabolism of mouse peritoneal macrophages by intracellular *Leishmania spp*, *Infect Immun*, 55, 587-593.

Carrera, L., Gazzinelli, R.T., Badolato, R., Hieny, S., Muller, W., Kuhn, R., and Sacks, D.L., 1996. *Leishmania* promastigotes selectively inhibit interleukin-12 induction in bone marrow-derived macrophages from susceptible and resistant mice, *J Exp Med*, 183, 515-526.

Carter, K.C., Dolan, T.F., Alexander, J., Baillie, A.J., and McColgan, C., 1989. Visceral leishmaniasis: Drug carrier system characteristics and the ability to clear parasites from the liver, spleen and bone marrow in *Leishmania donovani* infected BALB/c mice, *J Pharm Pharmacol*, 41(2), 87-91.

Castillo-Garit, J.A., Abad, C., Rodriguez-Borges, J.E., Marrero-Ponce, Y., and Torrens, F., 2012. A review of QSAR studies to discover new drug-like compounds actives against leishmaniasis and trypanosomiasis, *Curr Top Med Chem*, 12(8), 852-865.

Chakraborty, P., and Basu, M.K., 1997. *Leishmania* phagolysosome: Drug trafficking and protein sorting across the compartment, *Crit Rev Microbiol*, 23, 253-268.

Chakraborty, R., Mukherjee, S., Lu, H.C., McGwire, B.S., Chang, K.P., and Basu, M.K., 1996. Kinetics of entry of virulent and avirulent strains of *Leishmania donovani* into macrophages: A possible role of virulence molecules (gp63 and LPG). *J Parasitol*, 82, 632-635.

Chakravarty, J., and Sundar, S., 2010. Drug resistance in leishmaniasis, *J Glob Infect Dis,* 2(2), 167-176.

Chang, K.P., and Fong, D., 1988. In cytopathology of parasitic diseases, Pitman Books, London (*Ciba Foundation Symposium 99*), 113-137.

Chaurasia, M., Singh, P.K., Jaiswal, A.K., Kumar, A., Pawar, V.K., Dube, A., Paliwal, S.K., and Chourasia, M.K., 2016. Bioinspired calcium phosphate nanoparticles featuring as efficient carrier and prompter for macrophage intervention in experimental leishmaniasis, *Pharm Res*, 33(11), 2617-2629.

Chen, F., Zhu, Y., Wu, J., Huang, P., and Cui, D., 2012. Nanostructured calcium phosphates: Preparation and their application in biomedicine, *Nano Biomed Eng*, 4(1), 41-49.

Choudhury, G., and Chang, K.P., 1990. Molecular determinants of *Leishmania* virulence, *Ann Rev Microbiol*, 44, 499-529.

Costa Lima, S.A., Resende, M., Silvestre, R., Tavares, J., Quaissi, A., Lin, P.K.T., and daSilva, A.C., 2012. Characterization and evaluation of BNIPDaoct-loaded PLGA nanoparticles for visceral leishmaniasis: In vitro and in vivo studies, *Nanomed*, 7(12), 1839-1849.

Costa, J.M.L., Marsden, P.D., Llanos-cuentas, E.A., Netto, E.M., Carvalho, E.M., Barral, A., Rosa, A.C., Cuba, C.C., Magalhaes, A.V., and Baretto, A.C., 1986. Disseminated cutaneous leishmaniasis in a field clinic in Bahia, Brazil: A report of eight cases, *J Trop Med Hyg*, 89, 319.

DaSilva, R.P., Hall, F.B., Joiner, K.A., and Sacks, D.L., 1989. CR1, the C3b receptor, mediate binding of infective *Leishmania major* metacyclic promastigote to human macrophages, *J Immunol*, 1989, 143, 617-622.

DeVeer, M.J., Curtis, J.M., Baldwin, T.M., Didonato, J.A., Sexton, A., McConville, M.J., Handman, E., and Schofield, L., 2003. MYD88 is essential for clearance of *Leishmania major*: Possible role for lipophosphoglycan and toll-like receptor-2 signalling, *Eur J Immunol*, 33, 2822-2831.

Dong, Z., Qi, X., Xie, K., and Fidler, I.J., 1993. Protein tyrosine kinase inhibitors decrease induction of nitric oxide synthase activity in lipopolysaccharide responsive and lipopolysaccharide nonresponsive murine macrophages, *J Immunol*, 151, 2717-2724.

Dutta, M., Bandyopadhyay, R., and Basu, M.K., 1994. Neoglycosylated liposomes as efficient ligands for the evaluation of specific-sugar receptors on macrophages in health and in experimental leishmaniasis, *Parasitol*, 109, 139-147.

Espuelas, S., Plano, D., Nguewa, P., Font, M., Palop, J.A., Irache, J.M., and Sanmartín, C., 2012. Innovative lead compounds and formulation strategies as newer kinetoplastid therapies, *Curr Med Chem*, 19(25), 4259-4288.

Fortin, A., Dorlo, T.P.C., Hendrickx, S., and Maes, L., 2016. Pharmacokinetics and pharmacodynamics of oleylphosphocholine in a hamster model of visceral leishmaniasis, *J Antimicrob Chemother*, 71(7), 1892-1898.

Fortin, A., Hendrickx, S., Yardley, V., Cos, P., Jansen, H., and Maes, L., 2012. Efficacy and tolerability of oleylphosphocholine (OLPC) in a laboratory model of visceral leishmaniasis, *J Antimicrob Chemother*, 67(11), 2707-2712.

Fouladvand, M., Barazesh, A., and Tahmasebi, R., 2013. Evaluation of in vitroantileishmanial activity of curcumin and its derivatives "Gallium curcumin, Indian curcumin and diacethyl curcumin", *Eur Rev Med Pharmacol Sci*, 17(24), 3306-3308.

Ghosh, S., Kar, N., and Bera, T., 2016. Oleanolic acid loaded polylactic co-glycolic acid-vitamin E TPGS nanoparticles for the treatment of Leishmania donovani infected visceral leishmaniasis, *Int J Biol Macromol*, 93, 961-970.

Hernandez, L., Galvez, R., Montoya, A., Checa, R., Bello, A., Bosschaerts, T., Jansen, H., Rupérez, C., Fortin, A., and Miró, G., 2014. First study in efficacy and tolerability of a new alkylphosphocholine molecule (oleylphosphocholine-OIPC) in the treatment of canine leishmaniasis due to *Leishmania infantum*, *Parasitol Res*, 113(1), 157-164.

Iannello, D., Arena, A., Buemi, C., Calapi, M., Stassi, G., Gazzana, D., and Mastroeni, P., 2003. Differential induction of TNF-α and IL-18 in human peripheral blood mononuclear cells infected with *Leishmania major* or *Leishmania donovani*, *New Microbiol*, 26, 399-404.

Immordino, M.L., Dosio, F., and Cattel, L., 2006. Stealth liposomes: Review of the basic science, rationale, and clinical applications, existing and potential, *Int J Nanomed*, 1(3), 297-315.

Italia, J.L., Ravi Kumar, M.N.V., and Carter, K.C., 2012. Evaluating the potential of polyester nanoparticles for per oral delivery of amphotericin B in treating visceral leishmaniasis, *J Biomed Nanotechnol*, 8(4), 695-702.

Italia, J.L., Yahya, M.M., Singh, D., and Ravikumar, M.N.V., 2009. Biodegradable nanoparticles improve oral bioavailability of amphotericin B and show reduced nephrotoxicity compared to intravenous fungizone, *Pharm Res*, 26(6), 1324-1331.

James, S.L., and Hibbs, J.B.Jr., 1990. The role of nitrogen oxides as effector molecules of parasite killing, *Parasitol Today*, 6, 303-305.

James, S.L., and Nacy, C., 1993. Effector functions of activated macrophages against parasites, *Curr Opin Immunol*, 5, 518-523.

Jebali, A., and Kazemi, B., 2013. Nano-based antileishmanial agents; A toxicological study on nanoparticles for future treatment of cutaneous leishmaniasis, *Toxicol In Vitro* 27(6), 1896-1904.

Jha, T.K., 1983. Evaluation of allopurinol in the treatment of kala-azar occurring in the North Bihar, India, *Trans R Soc Trop Med Hyg*, 77, 204.

Jha, T.K., 1983. Evaluation of diamidine compound (pentamidine isethionate) in the treatment resistant cases of kala-azar occurring in North Bihar, India, *Trans R Soc Trop Med Hyg*, 77, 167.

Kager, P.A., Rees, P.H., Welde, B.T., Hockmeyer, W.T., and Lyerly, W.H., 1981. Allopurinol in the treatment of visceral leishmaniasis, *Trans Roy Soc Med Hyg*, 75, 556.

Keighobadi, M., Fakhar, M., and Emami, S., 2015. Hypothesis: the potential application of doxorubicin against cutaneous leishmaniasis, *Trop Parasitol*, 5(1), 69-70.

Kester, M., Heakal, Y., Fox, T., Sharma, A., Robertson, G.P., Morgan, T.T., Altinoğlu, E.I., Tabaković, A., Parette, M.R., Rouse, S.M., Ruiz-Velasco, V., and Adair, J.H., 2008. Calcium phosphate nanocomposite particles for in vitro imaging and encapsulated chemotherapeutic drug delivery to cancer cells, *Nano Lett*, 8(12), 4116-4121.

Kima, P.E., Ruddie, N.H., and McMahon-Pratt, D., 1997. Presentation via the class I pathway by *Leishmania amazonensis* infected macrophages of an endogenous leishmanial antigen to CD8+ T cells, *J Immunol*, 159, 1828-1834.

Kumar, R., Sahoo, G.C., Pandey, K., Das, V., and Das, P., 2015. Study the effects of PLGA-PEG encapsulated amphotericin B nanoparticle drug delivery system against *Leishmania donovani*, *Drug Deliv*, 22(3), 383-388.

Kumar, R., Sahoo, G.C., Pandey, K., Das, V.N.R., Topno, R.K., Ansari, M.Y., Rana, S., and Das, P., 2016. Development of PLGA-PEG encapsulated miltefosine based drug delivery system against visceral leishmaniasis, *Mater Sci Eng C Mater Biol Appl*, 59, 748-753.

Lala, S., Pramanick, S., Mukhopadhyay, S., Bandyopadhyay, S., and Basu, M.K., 2004. Harmine: Evaluation of its antileishmanial properties in various vesicular delivery systems, *J Drug Target*, 12(3), 165-175.

Lang, T., Prina, F., and Sibthorpe, D., 1997. Blackwell JM. Nramp1 transfection transfers Ity / Lsh / Bcg related pleiotropic effects on macrophage activation: Influence on antigen processing and presentation, *Infect Immun*, 65, 380-386.

Larabi, M., Yardley, V., Loiseau, P.M., Appel, M., Legrand, P., Gulik, A., Bories, C., Croft, S.L., and Barratt, G., 2003. Toxicity and antileishmanial activity of a new stable lipid suspension of amphotericin B, *Antimicrob Agents Chemother*, 47(12), 3774-3779.

Le Pape, P., 2008. Development of new antileishmanial drugs-current knowledge and future prospects, *J Enzyme Inhib med Chem*, 23(5), 708-718.

Liew, F.Y., Millott, S., Parkinson, C., Palmer, R.M., and Moncada, S., 1990. Macrophage killing of *Leishmania* parasite in vivo is mediated by nitric oxide from L-arginine, *J Immunol*, 144, 4794-4797.

Lin, J.Y., and Chadee, K., 1992. Macrophage cytotoxicity against *Entamoeba histolytica* trophozoites is mediated by nitric oxide from L-arginine, *J Immunol*, 148, 3999-4005.

Lu, H.G., Zhang, L., Chang, K.P., and Docampo, R., 1997. Intracellular Ca^{2+} pool content and signalling and expression of a calcium pump are linked to virulence in *Leishmania mexicana amazonensis* amastigotes, *J Biol Chem*, 272, 9464-9473.

Mandal, A.K., 2017. Copper nanomaterials as drug delivery system against infectious agents and cancerous cells, *J Appl Life Sci Int*, 15(4), 38444.

Mandal, A.K., 2017. Gold nanomaterials as drug delivery system against diseases, *Ann Res Rev Biol*, 19(6), 37866.

Mandal, A.K., 2017. Silver nanoparticles as drug delivery vehicle against infections, *Glob J Nanomed*, 3(2), 555607.

Marr, A.K., Mc Gwire, B.S., and Mc Master, W.R., 2012. Modes of action of leishmanicidal antimicrobial peptides, *Future Microbiol*, 7(9), 1047-1059.

Mattner, F., Padova, K.D., and Alber G., 1996. Interleukin-12 is indispensible for protective immunity against *Leishmania major*, *Infect Immunol*, 65, 4378-4383.

McDowell, M.A., and Sacks, D.L., 1999. Inhibition of host cell signal transduction by *Leishmania*: Observation relevant to the selective impairment of IL-12 response, *Curr Opin Microbiol*, 8, 438-444.

Mishra, B.B., Kale, R.R., Singh, R.K., and Tiwari, V.K., 2009. Alkaloids: Future prospective to combat leishmaniasis, *Fitoterapia*, 80(2), 81-90.

Mishra, J., Dey, A., Singh, N., Somvanshi, R., and Singh, S., 2013. Evaluation of toxicity and therapeutic efficacy of a new liposomal formulation of amphotericin B in a mouse model, *Ind J Med Res*, 137(4), 767-776.

Mishra, M., Sing, M.P., Choudhury, D., Singh, V.P., and Khan, A.B., 1991. Amphotericin B for second-line treatment of Indian kala-azar, *Lancet*, 337, 926.

Mitra, M., Mandal, A.K., Chatterjee, T.K., and Das, N., 2005. Targeting of mannosylated liposome incorporated benzyl derivative of *Penicillium nigricans* derived compound MT81 to reticuloendothelial systems for the treatment of visceral leishmaniasis, *J Drug Target*, 13(5), 285-293.

Moll, H., 1997. The role of chemokines and accessory cells in the immunoregulation of cutaneous leishmaniasis, *Behring Inst Mitteillungen*, 99, 73-78.

Mondal, S., Roy, P., Das, S., Halder, A., Mukherjee, A., and Bera, T., 2013. In vitro susceptibilities of wild and drug resistant *Leishmania donovani* amastigote stages to andrographolide nanoparticle: Role of vitamin E derivative TPGS for nanoparticle efficacy, *PLoS One*, 8(12), e81492.

Mukherjee, S., Ghosh, C., and Basu, M.K., 1988. Leishmania donovani: Role of microviscosity of macrophage membrane in the process of parasite attachment and utilization, *Exp Parasitol*, 66, 18-26.

Muller, I., Pedrazzini, T., Farrell, J.P., and Louis, J., 1989. T cell responses and immunity to experimental infection with *Leishmania major*, *Ann Rev Immunol*, 7, 561-578.

Nadhman, A., Nazir, S., Khan, M.I., Arooj, S., Bakhtiar, M., Shahnaz, G., and Yasinzai, M., 2014. PEGylated silver doped zinc oxide nanoparticles as novel photosensitizers for photodynamic therapy against *Leishmania*, *Free Radic Biol Med*, 77, 230-238.

Nadhman, A., Nazir, S., Khan, M.I., Ayub, A., Muhammad, B., Khan, M., Shams, D.F., and Yasinzai, M., 2015. Visible-light-responsive ZnCuO nanoparticles: Benign photodynamic killers of infectious protozoans, *Int J Nanomed*, 10, 6891-6903.

Nagle, A.S., Khare, S., Kumar, A.B., Supek, F., Buchynskyy, A., Mathison, C.J., Chennamaneni, N.K., Pendem, N., Buckner, F.S.,, Gelb, M.H., and Molteni, V., 2014. Recent developments in drug discovery for leishmaniasis and human African trypanosomiasis, *Chem Rev*, 114(22), 11305-11347.

Nahar, M., and Jain, N.K., 2009. Preparation, characterization and evaluation of targeting potential of amphotericin B-loaded engineered PLGA nanoparticles, *Pharm Res*, 26(12), 2588-2598.

Nandan, D., and Reinerb, N.E., 1995. Attenuation of gamma interferon induced tyrosine phosphorylation in mononuclear phagocytes infected with *Leishmania donovani*: Selective inhibition of signalling through Janus kinases and STAT1, *Infect Immunol*, 63, 4495-4500.

New, R.R.C., Chance, M.L., Thomas, S.C., and Peters, W., 1978. Antileishmanial activity of antimonials entrapped in liposomes, *Nature*, 272(5648), 55-56.

Norsworthy, N.B., Sun, J., Elnaiem, D., Lanzaro, G., and Soong, L., 2004. Sandfly saliva enhances *Leishmania amazonensis* infection by modulating interleukin-10 production, *Infect Immunol*, 72, 1240-1247.

Nussler, A.K., Billar, T.R., Liu, Z.Z., and Morris, S.M.Jr., 1994. Coinduction of nitric oxide synthase and argininosuccinate synthetase in a murine macrophage cell line, *J Biol Chem*, 269, 1257-1262.

Pal, S., Mandal, A., and Duttagupta, S., 2001. Studies on stibanate resistant *Leishmania donovani* isolates of Indian origin, *Ind J Exp Biol*, 39, 249-254.

Pei, Y., and Yeo, Y., 2016. Drug delivery to macrophages: Challenges and opportunities, *J Control Rel*. 240, 202-211.

Pena, S., Scarone, L., and Serra, G., 2015. Macrocycles as potential therapeutic agents in neglected diseases, *Future Med Chem*, 7(3), 355-382.

Peniche, A.G., Renslo, A.R., Melby, P.C., and Travi, B.L., 2015. Antileishmanial activity of disulfiram and thiuram disulfide analogs in an ex vivo model system is selectively enhanced by the addition of divalent metal ions, *Antimicrob Agents Chemother*, 59(10), 6463-6470.

Peters, C., Stierhof, V.D., and Ilg, T., 1997. Proteophosphoglycan secreted by *Leishmania Mexicana* amastigotes causes vacuole formation in macrophages, *Infect Immunol*, 65, 785-786.

Polonio, T., and Efferth, T., 2008. Leishmaniasis: Drug resistance and natural products (review), *Int J Mol Med*, 22(3), 277-286.

Prajapati, V.K., Awasthi, K, Yadav, T..P, Rai, M., Srivastava, O.N., and Sundar, S., 2012. An oral formulation of

amphotericin B attached to functionalized carbon nanotubes is an effective treatment for experimental visceral leishmaniasis, *J Infect Dis*, 205(2), 333-336.

Prajapati, V.K., Awasthi, K., Gautam, S., Yadav, T.P., Rai, M., Srivastava, O.N., and Sundar, S., 2011. Targeted killing of *Leishmania donovani* in vivo and in vitro with amphotericin B attached to functionalized carbon nanotubes, *J Antimicrob Chemother*, 66(4), 874-879.

Proulx, M.E., Desormeaux, A., Marquis, J.F., Olivier, M., and Bergeron, M.G., 2001. Treatment of visceral leishmaniasis with sterically stabilized liposomes containing camptothecin, *Antimicrob Agents Chemother*, 45(9), 2623-2627.

Ray, M., Gam, A.A., Boykins, R.A., and Kenney, R.T., 2000. Inhibition of interferon gamma signalling by *Leishmania donovani*, *J Infect Dis*, 181, 1121-1128.

Reiner, N.E., Ng, W., Ma, T., and McMaster, R.W., 1988. Kinetics of gamma interferon binding and induction of major histocompatability complex class II mRNA in *Leishmania* infected macrophages, *Proc Natl Acad Sci USA*, 85, 4330-4334.

Reiner, S.L., Zheng, S., Wang, Z.E., Stowing, L., and Locksley, R.M., 1994. Leishmania promastigotes evade interleukin 12 (IL-12) induction by macrophages and stimulate a broad range of cytokines from CD4+ T cells during initiation of infection, *J Exp Med*, 179, 447-456.

Rocha, R.A., Sampaio, R.N., Guerra, M., Magalhaes, A., Cuba, C.C., Barreto, A.C., and Marsden, P.D., 1980. Apparent glucantime failure in five patients with mucocutaneous leishmaniasis, *J Trop Med Hyg*, 83, 131.

Rosas, L.E., Keiser, T., Pyles, R., Durbin, J., and Satoskar, A.R., 2003. Development of protective immunity against cutaneous leishmaniasis is dependent on STAT1 mediated IFNγ signalling pathway, *Eur J Immunol*, 33, 1799-1805.

Rossi, D., and Zlotkin, A., 2000. The biology of chemokines and their receptors, *Ann Rev Immunol*, 18, 217-242.

Roy, P., Das, S., Bera, T., Mondal, S., and Mukherjee, A., 2010. Andrographolide nanoparticles in leishmaniasis: Characterization and in vitro evaluations, *Int J Nanomed*, 5(1), 1113-1121.

Roychoudhury, J., Sinha, R., and Ali, N., 2011. Therapy with sodium stibogluconate in stearylamine-bearing liposomes confers cure against SSG-resistant *Leishmania donovani* in BALB/c mice, *PLoS One*, 6(3), e 17376.

Roychoudhury, K., and Roy, S., 2004. Role of chemokines in *Leishmania* infection, *Curr Mol Med*, 4, 691-696.

Sana, S., Ghosh, S., Das, N., Sarkar, S., and Mandal, A.K., 2017. Vesicular melatonin efficiently downregulates sodium fluoride-induced rat hepato- and broncho- TNF-α, TGF-β expressions, and associated oxidative injury: A comparative study of liposomal and nanoencapsulated forms, *Int J Nanomedicine*, 12, 4059-4071.

Sangshetti, J.N., Kalam Khan, F.A., Kulkarni, A.A., Arote, R., and Patil, R.H., 2015. Antileishmanial drug discovery: Comprehensive review of the last 10 years, *RSC Adv*, 5(41), 32376-32415.

Saudagar, P., and Dubey, V.K., 2014. Carbon nanotube based betulin formulation shows better efficacy against *Leishmania* parasite, *Parasitol Int*, 63(6), 772-776.

Shadab, M., Jha, B., Asad, M., Deepthi, M., Kamran, M., and Ali, N., 2017. Apoptosis-like cell death in *Leishmania donovani* treated with Kalsome^{TM}10, a new liposomal amphotericin B, *PLoS One* 12(2), e 0171306.

Silva, T., Abengozar, M.A., Fernandez-Reyes, M., Andreu, D., Nazmi, K., Bolscher, J.G., Bastos, M., and Rivas, L., 2012. Enhanced leishmanicidal activity of cryptopeptide chimeras from the active N1 domain of bovine lactoferrin, *Amino Acids*, 43(6), 2265-2277.

Sinha, J., Mukhopadhyay, S., Das, N., and Basu, M.K., 2000. Targeting of liposomal andrographolide to *L. donovani*-infected macrophages in vivo, *Drug Deliv*, 7, 209-213.

Sinha, J., Raay, B., Das, N., Medda, S., Garai, S., Mahato, S.B., and Basu, M.K., 2002. Bacopasaponin C: Critical evaluation of anti-leishmanial properties in various delivery modes, *Drug Deliv*, 9, 55-62.

Stobie, L., Gurunathan, S., Prussion, C., Sacks, D.L., Glaichenhaus, N., Wu, C.Y., and Seder, R.A., 2000. The role of antigen and IL-12 in sustaining Th1 memory cells in vivo: IL-12 is required to maintain memory / effector Th1 cells sufficient to mediate protection to an infectious parasite challemge, *Proc Natl Acad Sci USA*, 97, 8427-8432.

Sundar, S., and Chakravarty, J., 2015. Investigational drugs for visceral leishmaniasis, *Expert Opin Investig Drugs*, 24(1), 43-59.

Tariq, A., Adnan, M., Amber, R., Pan, K., Mussarat, S., and Shinwari, Z.K., 2016. Ethnomedicines and anti-parasitic activities of Pakistani medicinal plants against *Plasmodium* and *Leishmania* parasites, *Ann Clin Microbiol Antimicrob*, 15(1), 52.

Tavares, J., Ouaissi, A., Silva, A.M., Lin, P.K.T., Roy, N., and Cordeiro-da-Silva, A., 2012. Anti-leishmanial activity of the bisnaphthalimidopropyl derivatives, *Parasitol Int*, 61(2), 360-363.

Thakur, C.P., Sinha, G.P., Pandey, A.K., Kumar, N., Kumar, P., Hassan, S.M., Narain, S., and Roy, A.K., 1998. Do the diminishing efficacy and increasing toxicity of sodium stibogluconate in the treatment of visceral leishmaniasis in Bihar, India, justify its continued use as a first-line drug? An observational study of 80 cases, *Ann Trop Med Parasitol*, 5, 561.

Tiwari, B., Pahuja, R., Kumar, P., Rath, S.K., Gupta, K.C., and Goyal, N., 2017. Nanotized curcumin and miltefosine, a potential combination for treatment of experimental visceral leishmaniasis, *Antimicrob Agents Chemother*, 61(3), e01169-16.

Torrado, J.J., Serrano, D.R., and Uchegbu, I.F., 2013. The oral delivery of amphotericin B, *Ther Deliv*, 4(1), 9-12.

Van De Ven, H., Paulussen, C., Feijens, P.B., Matheeussen, A., Rombaut, P., Kayaert, P., Van den Mooter, G., Weyenberg, W., Cos, P., Maes, L., and Ludwig A., 2012. PLGA nanoparticles and nanosuspensions with amphotericin B: Potent in vitro and in vivo alternatives to Fungizone and AmBisome, *J Control Rel*, 161(3), 795-803.

Vouldoukis, I., Beeherel, P.A., Riveros-Moreao, V., Arock, M., daSilva, O., Dehre, P., Mazier, D., and Mossalayi, M.D., 1997. Interleukin-10 and Interleukin-4 inhibit intracellular killing of *Leishmania infantum* and *Leishmania major* by human macrophages by decreasing nitric oxide generation, *Eur J Immunol*, 27, 860-865.

Want, M.Y., Islamuddin, M., Chouhan, G., Dasgupta, A.K., Chattppadhyay, A.P., and Afrin, F., 2014. A new approach for the delivery of artemisinin: Formulation, characterization, and ex-vivo antileishmanial studies, *J Colloid Interface Sci*, 432, 258-269.

Wright, S.D., and Silverstein, S.C., 1982. Tumor-promoting phorbol esters stimulate C3b and C3b´ receptor-mediated phagocytosis in cultured human monocytes, *J Exp Med*, 156, 1149-1164.

Yang, D.M., and Liew, F.Y., 1993. Effects of qinghaosu (artemisinin) and its derivatives on experimental cutaneous leishmaniasis, *Parasitol*, 106(1), 7-11.

Zhang, Z., Tan, S., and Feng, S.S., 2012. Vitamin E TPGS as a molecular biomaterial for drug delivery. *Biomaterials*, 33(19), 4889-4906.

****