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Emerging therapeutic targets for treatment of leishmaniasis

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Abstract

Introduction—Parasitic diseases that pose a threat to human life include leishmaniasis – caused by protozoan parasite *Leishmania* species. Existing drugs have limitations due to deleterious side effects like teratogenicity, high cost and drug resistance. These call for the need to have an insight into the therapeutic aspects of disease.

Areas covered—We have identified different drug targets, *via*. molecular, immunological, metabolic as well as by system biology approaches. We bring these promising drug targets into light so that they can be explored to their maximum. In an effort to bridge the gaps between existing knowledge and future prospects of drug discovery, we have compiled interesting studies on drug targets thereby paving the way for establishment of better therapeutic aspects.

Expert opinion—Advancements in technology sheds light on many unexplored pathways. Further probing of well established pathways, led to the discovery of new drug targets. This review is a comprehensive report on current and emerging drug targets, with emphasis on several metabolic targets, organellar biochemistry, salvage pathways, epigenetics, kinome, etc. Identification of new targets can contribute significantly towards strengthening the pipeline for disease elimination.

Keywords

leishmaniasis; drug discovery; drug targets; mitochondria; metabolic pathways

Introduction

Leishmaniasis is a group of manifestations ranging from cutaneous to most severe visceral forms, caused by protozoan parasite *Leishmania* species. Visceral form of disease, caused by *Leishmania donovani*, characterised by prolonged fever (>2 weeks), weight loss, anemia, and splenomegaly. India, Bangladesh, Sudan, South Sudan, Brazil and Ethiopia together contribute for more than 90% of the global disease burden with 0.3 million new VL cases reported each year. *Leishmania* parasites cycle between two hosts- human and sandfly, in two distinct life stages, as flagellar promastigote or amastigote form. The promastigotes

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Declaration of Interest

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from sandfly are injected into the human upon blood feeding where they are phagocytosed by circulating monocytes, dendritic cells and/or neutrophils. Once inside the phagosome these parasites undergo differentiation to non-flagellar amastigote. These amastigotes divide several times until bursting of host cells to infect another cell.

There was a collaborative association of the governments of India, Bangladesh and Nepal for elimination of this disease in 2005[1, 2]. This memorandum was later renewed in 2014, upon consideration of several underestimated parameters while reporting new cases. With the main focus on early case detection, diagnosis, treatment, as well as vector management. VL elimination program has been extended to 2020 with the establishment of new taskforce to facilitate attainment of the set goal [2]. There are several challenges to make the target a realistic goal including the drug resistance, toxicity issues related with current therapeutic options, asymptomatic carriers, inadequate knowledge on vector biome and non-availability of vaccine. Therefore, coordinated monitoring at different levels of implementation, proper case management, social awareness, active case detection and strong partnership among stakeholders could serve to make the way easy.

Recent years have experienced changes in terms of flow of funds to support drug discovery. The complex life cycle of the parasite also includes several checkpoints that might be exploited towards drug development. Additionally, technological advances in the field of clinical research and the availability of complete genome sequence of *Leishmania* [4], have further strengthened the way towards the drug discovery. This review is an effort towards comprehensive understanding of current chemotherapeutic options, its limitations and recent developments in drug designing and discovery. An insight into the proteins from different biological pathways attribute towards the identification of potential drug targets.

Current therapeutic option

The current treatment option for leishmaniasis relies solely on chemotherapy, indeed, the lack of effective and affordable drugs has led the attention of the scientific community towards drug research and development of therapeutic options. The WHO approved treatment regimen for antimonial was a 30-day treatment that costed between 120USD to 150USD with cost effective disease intervention strategy [5-7]. However, later the long hospitalization period, cardiotoxicity [8], cirrhosis, pancreatic toxicity [9] and emergence of high proportions of drug resistant cases [10-12] led to the use of pentamidine in early 1980s as a second line therapy for refractory cases but it remained unaffordable for most patients. The high costs and toxicity issues raised public health concerns for safe and cost effective option [13], leading towards the emergence of amphotericin B and its lipid formulation as the second line therapy. However, the failure of antimonials and pentamidine along with the emergence of drug resistance made amphotericin B as the first line drug in Bihar in 1990s.

Indeed, limited number of registered drugs for leishmaniasis with the high costs of treatment, toxicity and drug resistance remain significant challenges for health authorities. This led to the concept of drug repurposing, where the clinically approved drugs used for treatment of other diseases can be used for leishmaniasis. Repurposed drugs included the conventional drugs- amphotericin B, paromomycin, and miltefosine. Amphotericin B

deoxycholate (AmBD) has been used as antifungal agent later used for treatment of visceral leishmaniasis (VL) in India [14, 15]. The liposomal formulation (AmBisome; Gilead Sciences) of the drug has been efficacious for several fungal infections and beneficial in patients with renal impairment as well as neutropenia [16], later in 1997 it was approved for the treatment of leishmaniasis [17].

Miltefosine, only approved oral drug for leishmaniasis [18] was originally discovered for its anti-cancer properties [19, 20]. However, its use was limited due to the high cost, teratogenic potential and differential drug susceptibility in different clinical isolates [21]. Paromomycin, a broad spectrum aminoglycoside antibiotic, has been used for treatment of bacterial infections, also found effective against protozoal infections as giardiasis, amoebiasis [22] and later against leishmaniasis in 1960s [23-25] It is approved in India for the treatment of VL. However, being aminoglycoside, it poses the risk of development of drug resistance, if used as monotherapy. Drug repurposing has also provided us with delamanid, approved anti-tubercular drug, with good efficacy in experimental leishmaniasis [26-28]. Multi-centre phase III trial suggested for use of paromomycin as registered drug for VL in India [29], but its efficacy was poor in Sudanese population where parasite clearance was below 50% [29]. Sitamaquine was another orally administrable drug with good efficacy in Indian[30] and Kenyan[31] population but significant nephrotoxicity, associated with the drug, abandoned its use. The growing issue of drug resistance called upon the use of combination therapy (miltefosine, antimonials, AmB and paromomycin in different combinations) which has been known to act synergistically and efficacious at lower doses.

In spite of the large population suffering from disease, the funds for the research and development of treatment options has been limited. One or the other limitations associated with current chemotherapeutic options calls for an urgent need for development of newer therapeutic options. Therefore, significant research in the field of therapeutic development has led towards the study of several target molecules for disease intervention.

Novel therapeutic options and drug discovery

Despite the remarkable researches for development of drug targets, chemotherapy remains to be the mainstay of successful treatment. As leishmaniasis affects poorest of poor with meagre returns on investments, pharmaceutical industry has low interest in development of new antileishmanial drugs. In 2000, only 0.1% contribution in health research was reported for malaria, leishmaniasis, trypanosomiasis and tuberculosis, while the estimated global burden for these diseases was 5% [32]. A sense of urgency for disease management led the policymakers to frame objective for assessment of new options. The drug discovery process from identification of active compound to its establishment as clinical candidate has been an arduous task; however, it remains to be the main goal of VL elimination programme. This section of the review aims to look into the researches in determining the novel candidates as drug targets.

Ongoing developments:Phytoproducts/Compounds as therapeutic agents

Phytotherapy is the study of the use of extracts from natural origin as medicines or health promoting agents. There has been growing interest in utilization of medicinal plants for treatment of different diseases [33]. Medicinal plants have further been a choice amongst general masses as a safe and inexpensive option for different ailments.

Historically, use of medicines for treating different diseases began with the use of herbs. During ancient times, in Mediterranean civilization, healing was associated with the use of herbs. Later advances in chemical sciences provided a platform for isolation and extraction of components of plant origin. Phytomedicines are the medicinal products derived from the plant extracts. These phytomedicines usually have complex chemical composition attributed to the presence of chemical groups including-alkaloids, phenylpropanoid, flavonoids, terpenoids and sterols [34-36]. Phytotherapy, these days is flourishing in search for low cost anti-leishmanial agents with fewer side effects and chemical diversity of plant extracts make them pharmacologically relevant for use as drug. Many research communities have discovered anti-parasitic drugs, however, leishmaniasis has received less attention. Therefore, search of novel therapeutic options remains to be of high priority for treating this neglected tropical disease. In last few decades, many new evidences for the use of medicinal plants for treatment of leishmaniasis have been reported. The noteworthy researches in this field remain to be the use of *Kalanchoe pinnata*, with triterpenes, sterols and flavonoids as the major constituents [37]; table 1 summarizes the major secondary metabolites/compounds with potent antileishmanial activity. The leishmanicidal activity was reported [38] to be similar to the meglumine antimoniate upon oral administration without any toxic side effects. The anti-parasitic activity has been attributed to macrophage mediated elevated levels of nitric oxide [39]. Studies from human CL model have reported significant reduction in lesion size without any alteration in serum transaminases, urea, alkaline phosphatase[40].

Naphthoquinone are another class of secondary metabolite from plant with potential anti-leishmanial activity. Plumbagin, naphthoquinone has been reported to inhibit trypanothione reductase from *L.donovani* and induces mitochondria mediated cell death [41]. Aloe vera leaf exudate has also been established to have potential antileishmanial activity against different *Leishmania* spp. by triggering programmed cell death [42]. The list of antileishmanial compounds does not end here; there are plethoras of plant derived products/ chemical compounds which have shown potential antileishmanial activity that remains beyond the scope of current article.

Nanotechnology based therapeutic options

In recent years nanotechnology has revolutionized the field of drug designing and development. It has been a promising tool in parasitic diseases where the parasite recrudescence against conventional chemotherapeutic options. Nanotechnology has provided a platform for drug development in two ways: design of drug delivery systems and nano-formulation of drugs which are easy targets for phagocytosis by macrophages therefore, causing targeted delivery of drug. Nanotechnology has managed to invent nanoparticles for drug delivery as carriers, liposomal formulation of amphotericin B reduces its toxicity

profile [43] Their biodegradability and non-immunogenic properties make them suitable candidate for research and therapeutic applications. Initially, nanotechnology approach for drug delivery used nanodisks impregnated with amphotericin B, polymeric nanoparticles loaded with pentamidine etc. Gold nanoparticles conjugated with quercetin was found to effective in sodium stibogluconate and paramomycin resistant parasites [44]. Nanotechnology has further improved the conventional leishmanization approach utilizing the liposome-protamine-DNA nanoparticle with immunostimulatory CpG for inducing T_H1 type immune response in BALB/c during *L.major* infection [45]. On the other hand, the conventional autoclaved parasite formulation of vaccine has been modified with the nano-formulation, where autoclaved *L.major* with PLGA and CpG showed highest IgG2a/IgG1 ratio and IFN- γ production induced strong immune responses against parasite in BALB/c mice [46]. Also, there are several micro- and nano-immunostimulatory adjuvants which have been used to enhance vaccine delivery and served as candidate leishmaniasis vaccine [47]. In this context, plasmid DNA encoding KMP-11 with poly(lactic-co-glycolic acid)(PLGA) nanoparticle elicited strong cellular and innate immune responses in the form of high levels of IFN- γ and TNF- α and significantly decreased parasite loads at infection site therefore, encouraging nano-based systems for vaccine development in mice model enhancing innate immune responses [48, 49]. Additionally, chitosan nanoparticles in combination with superoxide dismutase increased T_H1 immune response therefore, providing a great option for development of single dose nanovaccine against leishmania infection [50]. One of the remarkable breakthroughs has been the high efficacy of nanoformulation of amphotericin-B with good safety profile and low production cost establishing it as a better alternative to conventional amphotericin B [51]. Recently, amphotericin B formulation with engineered poly (propylene-imine) dendrimeric nanoconjugate has been reported to have higher parasiticidal activity with reduced toxicity for macrophages as reported from *in vitro* and *in vivo* assays [52]. Nanoliposomal formulation of 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) with soluble leishmania antigen (SLA) has been documented to enhance T_H1 type of immune response and induces protection against *L.major* infection. Similarly, engineered DOTAP has been reported as conjugated nanoparticle with amastigote class I nuclease, second generation vaccine candidate improved the T_H1 response and therefore, serving as promising candidate for vaccine development against CL [53]. Thus, nanotechnology has illuminated the way towards vaccine and drug development by improving its therapeutic proficiency.

Ongoing developments and emerging targets

Metabolic pathways as therapeutic target

Current chemotherapeutic drugs are known to target various metabolic pathways. Pentavalent antimonials interfere with DNA replication, fatty acid oxidation, ADP phosphorylation and glycolysis. Amphotericin B acts to target ergosterol in parasite cell membrane. Miltefosine induces apoptosis, paramomycin inhibits cytochrome C in mitochondria and pentamidine reduces membrane potential and inhibits topoisomerase in mitochondria. However, currently, available options suffer from limitations of high cost, side effects and drug resistance and calls for alternative options. The conventional drugs target different biomolecules, indeed, drugs with greatest efficacy are directed against protein

targets [54]. Indeed, the major concern for therapeutic target remains the identification of degree of homology between the host and parasite proteins and therefore, selecting inhibitors which reacts with parasite protein without damaging the host system [55]. The drugs directed against the energy metabolism remained to be the choice that included many protein targets for therapeutics. Figure 1 provides an overview of five decades of studies targeting different metabolic pathways in *Leishmania*. Glycosome and mitochondria are main energy production houses being the sites for glycolysis and Krebs's cycle as well as oxidative phosphorylation, respectively.

Indeed, blocking of any step of glycolysis causes an arrest of the glycolytic influx and thus, parasite killing. This was also demonstrated in *T.brucei* where glucose starvation or incubation with glucose transporter inhibitor led to parasite death within minutes. Similarly, pentalactone or bromopyruvate, inhibitors of glyceraldehyde-3-phosphate dehydrogenase and inhibitors of pyruvate killed the parasite but these studies suffered limitation since, these inhibitors blocked the human counterpart. Despite the major contribution of mitochondria-mediated breakdown of fatty acids in *Leishmania* life cycle, glycolysis remains an important process. Structure of enolase has not been deciphered while few atypical residues have reported which could serve as drug target. Pyruvate kinase structure has been determined in *L.mexicana* with unambiguous selectivity feature which makes it a candidate for drug targeting. Similarly, glyceraldehyde-3-phosphate dehydrogenase has been depicted with 30% homology with human counterpart making it an important candidate for drug designing. Most of the enzymes of glycolytic pathways are localised inside glycosomes and this compartmentalisation promotes parasite survival, and thus, inhibitors designed for preventing their transport by blocking membrane transporters responsible for glucose flux through the glycosome can serve as a drug target.

Mitochondria, vital organ for parasite survival, serves as target for several drugs. The insight into the mechanism of action of drugs emphasises the role of this organelle as a key determinant for disease pathogenesis. The proteins in mitochondria have been derived from two sources- nucleus and small part encoded by mitochondria itself. Currently available studies on trypanosomatid mitochondria are scarce, however, the available set of findings established the essential role for this organelle and its peculiarities as compared to human counterpart making it an attractive candidate for drug development. Experimental evidences suggest mitochondria targeting by conventional chemotherapeutic approaches. Amphotericin B results in membrane permeability and rapid decline in mitochondrial membrane potential, likewise, pentamidine also destroys membrane potential [56], miltefosine inhibits cytochrome C oxidase [57]. On the other hand, *Leishmania* transmembrane redox system differs from mammalian cells in being less sensitive to chloroquine and more sensitive towards niclosamide [58] therefore, suggesting the possibility of membrane electron transport and proton pumping to act as an attractive therapeutic target.

A number of researches for the antileishmanial agents led to establishment of chalcones as potential drug which targets ultrastructure and functions of mitochondria [59, 60], later its ability to inhibit fumarate reductase[61] has established it as potential drug target. Endochin-like quinolones (ELQs) has been another potent inhibitor of cytochrome bc 1 in *Plasmodium* [62]. It has been shown to have toxic effects on amastigotes of *L.donovani* and *L.mexicana*,

however, hydroxynaphthoquinone buparvaquone acts as more potent inhibitor of electron transport, ATP production and parasite growth [63] raising concern for targeting cytochrome bc1 as potential therapeutic option.

Other inhibitors of mitochondria included benzophenone-derived bisphosphonium salt which targeted complex II [64]; and anti-malarial compound artemisinin, have shown anti-leishmanial activity by inducing apoptosis [65] Hydroxynaphthoquinone atovaquone inhibits *L.infantum* [66]; tafenoquine, analogue of primaquine, causes mitochondrial dysfunction by inhibiting cytochrome c reductase (complex III) decreasing oxygen consumption and mitochondrial membrane potential and ultimately apoptosis [67].

Mitochondria remains to be the principal site for fatty acid metabolism where 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase from *L.donovani* [68] has been established as potential drug target. Similarly another drug target, fatty acyl-CoA ligase regulates cellular homeostasis of lipid, differentially regulated in antimony resistant *L.donovani* [69] affirming its potential as a drug target. Sterol biosynthesis has been important constituent for cellular functions and maintenance of cell structure. The major sterols in trypanosomatids are ergosterol and 24-methyl sterol, essential for growth and viability. This makes, sterol and fatty acid metabolic pathway, attractive drug targets. Several other enzymes involved in sterol biosynthesis has been reported for their potential as drug target including- squalene synthase [70], squalene epoxide [71], farnesyl diphosphate synthase [72, 73], sterol methyl transferase[74, 75], sterol 14 alpha demethylase[76, 77] and many more as demonstrated in different chemical inhibition studies. Recent reports have suggested combination targeting where two steps in sterol biosynthesis are targeted at once using imipramine and miconazole[78].

Edelfosine has recently been demonstrated as target drug for disrupting the mitochondrial membrane potential by recruiting F0-F1 ATPase in the lipid raft, driving DNA disruption and thus serving as novel drug target for the treatment of leishmaniasis[79].

Polyamine metabolism remains another important candidate pathway for the drug designing. Polyamines are strongly associated with cell survival, growth and proliferation. Arginase is the first enzyme in polyamine biosynthetic pathway [80]. Other enzyme as potent drug targets include ornithine decarboxylase[81, 82], S-adenosylmethionine decarboxylase(S-AdoMet inhibitor CGP40215A, 1996) [83], spermidine synthase [84], trypanothione synthetase [85, 86], trypanothione reductase [87], tryparedoxin peroxidase [88], deoxyhypusine synthase [89], and deoxyhypusine hydroxylase[90].Polyamine transporters-putrescine transporters, DAB, LmPOT1 etc. [91-93].

Folate metabolism constitutes another crucial biochemical pathway essential for parasite survival [94]. The potential drug targets from folate pathway include pteridine reductase, dihydrofolate reductase-thymidylate synthase [95, 96], folylpolyglutamate synthetase [97] and serine hydroxymethyl transferase [98].

Antimicrobial peptides as therapeutic target

Antimicrobial peptides (AMPs) are multifunctional, cationic protein weapons of innate immune system in wide range of species. These are usually expressed at low levels but upregulated upon infection or inflammation [99]. It constitutes the most primitive but important immune defense armory because of its rapid action, broad target range, amphipathicity and flexibility of utilization in conjunction with existing regimens [100]. The AMPs serves many functions as chemotactic agents for leukocytes, interact with the microbial membrane inducing autophagic [101], necrotic or apoptotic cell death [102]. These are structurally diverse peptides, with high content of basic amino acid residues while certain subsets are also rich in cysteine allowing for their functionality[103]. In spite of its wellknown role to destabilize the membrane, it also penetrates intracellular organelles and has pleiotropic effect on bioenergetic function of cells [104, 105]. Several AMPs have antileishmanial activity including dermaseptin[106], phylloseptin [107], bombinins[108], temporins [109], spinigerin [110] and magainins[111]. Other class of AMPs expressed in mammals include cathelicidin (Protegrin-1,SMAP-18,-27) [101] and defensin [112] which influence host inflammatory responses by serving as chemokines or inducing chemokine secretion by other cells leading to migration of neutrophils, monocytes and macrophages. Chemokines have also been known for their antimicrobial peptide activity on pathogens termed as kinocidins [113-115]. Further, several other AMPs from different species have been known to effect *Leishmania* [116]. However, the clever parasite overcomes this innate barrier of AMPs by employing leishmanolysin (gp63) as documented from knock-out studies in mice [117]. It is unlikely that leishmanolysin has significant effect on *Leishmania* resistance against AMPs as the parasite resides in phagolysosome. These AMPs have been known for inducing cell death in the parasite using different mechanisms. These AMPs break the membrane potential, equilibrate the internal as well as the external pH without perturbing the cell membrane and alters ATP content as documented from *in vitro* as well as *in vivo* studies [105, 111, 118]. The AMPs have less activity in LPG-deficient as well as late stationary phase parasites (LPG enriched) suggestive of the LPG mediated alteration in the membrane as regulator of AMP activity. They induce formation of vacuoles without any alteration in their activities upon caspase-blocking indicating for their ability to cause autophagy mediated cell death in parasites [101]. While other class of AMPs from human salivary gland causes disruption of mitochondrial membrane potential, decreased oxygen consumption and ATP depletion[119]. Therefore, it is well understood that different classes of AMPs disrupts parasite membrane, target mitochondria [119] and delocalize intracellular calcium crucial for inducing cell death by affecting mitochondrial activity. Intracellular AMPs affect calcium reserves in parasites (acidocalcisomes, glycosomes and/or endoplasmic reticulum) [120]. These researches have provided reasonable basis for the development of AMP based therapeutic targets as suggested from the preliminary evidences from animal studies [112, 121].

However, there are a number of AMPs which have been screened against *Leishmania*, the picture of development of AMPs based anti-leishmanial agent still remains elusive because of limited studies on amastigote form of parasite and intracellular nature of the parasite making it difficult for access by AMPs. Additionally, given the differences in surface

architecture of two forms of parasite, there always remains a need for developing/screening AMPs against the amastigotes. However, studies from mice model reported the absence of cathelicidin-AMP associated with exacerbated infection [122], therefore, augmentation of intracellular AMP expression may serve as novel therapeutic approach. AMPs can also be targeted in the vector where parasite grows and differentiates [123] so, understanding of role of vector AMPs in parasite dissemination remains a crucial checkpoint for intervention of parasite transmission from insect to humans and therefore, this could serve as a major breakthrough in controlling the number of new cases and asymptomatic carriers of disease.

Proteasome and cell cycle as therapeutic target

Proteasome is multi-subunit protein complex in the cytoplasm and nucleolus, which regulates cellular protein turnover and degradation of misfolded proteins. It has two main components- the core particle and the regulatory subunit. Proteins destined for degradation are conjugated with ubiquitin (in eukaryotes) or ubiquitin-like protein (pup) (in prokaryotes) [124]. These proteins are then recognised by regulatory proteins flanking the proteolytic core of proteasome. These proteins then unfold polyubiquitinated proteins, threading it through the narrow opening of the core subunit upon the removal of ubiquitin and pup chains [125] [126]. The ubiquitin-proteasome system from protozoans has been widely studied [127]. The study of proteasome as drug target dates back to the 90s when use of proteasome inhibitors altered the cell growth, development and differentiation of the parasite in concentration-dependent manner [128]. *In vitro* assays in *L.donovani* with 2 1M MG-132 or 5 1M suggested for induction of programmed cell death in parasite while the surviving parasites were short with rounded apical ends and damaged mitochondria [129]. Treatment with conventional proteasome inhibitor, lactacystin, caused sharp decline in the parasite viability in the macrophages [130]. Phenotypic screening of three million compounds and hit-to-lead optimization against *Leishmania donovani*, *Trypanosoma cruzi* and *Trypanosoma brucei* identified efficacious compound for VL, CL, Chagas disease and trypanosomiasis targeting parasite proteasome [131]. A high-throughput proteomic study at Genomics Institute of the Novartis Research Foundation (GNF), led to the identification of a compound termed GNF5343, GNF6702 with good efficacy for *L.donovani*, *T.brucei* and *T.cruzi* cultures [131].

There are reports from mitochondrial protein, Ufm1 from *Leishmania*, has been suggested as a protein drug target [132] which has shown remarkable similarities between mammalian and *Leishmania* conjugation system. This has fueled the idea for use of anticancer drugs that target ubiquitin pathway as a key for development of the antiparasitic drug which has been suggested as protein drug target. The anti-parasitic effect of disruption of this pathway leads to decreased parasite survival therefore, increasing its recognition as a potential therapeutic tool. Deubiquitinases are another key regulator of ubiquitin-proteasome pathway, exploited as anti-cancer weapon has further been of interest for development of antileishmanial drug because of the ability of the parasite to obstruct host protein degradation system for its survival [133] provides impetus for utilizing this pathway as therapeutic option.

There are varieties of diseases with defective protein kinase signaling, which is usually required for progression of cell cycle therefore, paving the way for use of specific inhibitors

to target these molecules for drug development. *Leishmania* has tightly controlled cell cycle program regulated by cyclin-dependent kinases (CDKs). Several small chemicals targeting CDKs are currently under clinical trial including alvocidib [134], seliciclib [135] etc. Due to their pivotal role in cell cycle these proteins serves as attractive target for drug discovery. The studies from mammalian and parasite system have revealed for many homologues in parasites e.g. CRK3 [136]. The protein kinases from cyclin-dependent kinase, cAMP-dependent kinase and mitogen activated protein kinase families are major target molecules from the parasite.

CRK3 in *Leishmania* is essential for proliferation and has been targeted for drug development with the use of chemical inhibitors [137]. The report from other cell cycle target from *Leishmania* is aurora kinases, essential for cell division has been established by bioinformatic as well as immunoproteomic approaches as drug target whose inhibition led to aberrant changes in cell cycle progression and parasite viability [138].

Histone acetyl transferase and histone deacetylases are another group of important molecules required for regulating gene transcription, cell cycle progression and essential for parasite survival. It has been reported to have therapeutic potential by pharmacological studies [139]. SIR2 family proteins are another interesting candidate for parasite survival and stage specific as documented in genetic deletion studies [140]. Recent findings on *Leishmania* glycogen synthase have revealed for its role in cell cycle progression therefore, drugs targeting this enzyme has therapeutic potential [141].

Metacaspases, known for their apoptotic function remains one the key components of the actively replicating amastigote and promastigote. However, its overexpression has been associated with growth retardation, changes in ploidy and impaired cytokinesis. Indeed, essentiality of metacaspases for segregation of the nucleus and kinetoplast apart from its role in programmed cell death, and absence of mammalian counterpart [142] makes this enzyme potential candidate with therapeutic application.

Secretory proteins/secretion pathway based therapeutic targets

Leishmania secretes a wide array of proteins in the extracellular milieu to cope with the hostile macrophage environment [143-146]. These secretory proteins are trafficked through eukaryotic secretion pathways where proteins are folded in endoplasmic reticulum and transported through the golgi for secretion outside the cell. So, processing and secretion of virulence factors remains crucial for parasite survival in the host [147].

Endoplasmic reticulum (ER) remains the key organelle for the secretory pathway, where the folding and packaging of the proteins is done for its delivery to respective sites. It also serves as the check point for the misfolded proteins which then is destined for the ubiquitin-proteasome system for their degradation.

Any kind of insult to ER activates unfolded protein response (UPR), which leads to activation of transmembrane proteins-Atf6, Ire1 and PERK. Computational modeling analysis revealed the involvement of PERK in *L. donovani* infection without measureable changes in the UPR-specific expression of BiP (ER chaperone) and increased sensitivity

towards ER stress-inducing drugs as compared to host macrophage, and these findings indicate ER stress as a pathway of therapeutic interest [148]. In addition to the UPR, there is extremely well coordinated quality control machinery of ubiquitin-proteasome system in cytosol termed as the ER associated degradation (ERAD), which degrades misfolded peptides upon extraction from ER [149]. However, the protozoan parasites contain minimal ERAD network therefore, these parasites are highly sensitive to inhibition of protein quality control system components. Recently, ER stress on *L.amazonensis* infection was associated with TLR2 dependent XBP1 formation that ultimately induced IFN- β expression and altered the oxidative response of infected macrophages causing parasite proliferation [150]. Thus, targeting this transcription factor can have potential therapeutic implications.

The PERK/ATF4 pathway plays an important role in protein homeostasis and is exploited by the parasite to establish infection. PERK signalling also diminishes translation and induces ATF4 expression which drives cell survival or induces cell death. PERK/eIF2 α /ATF4 signaling is known to induce upregulation of cytoprotective autophagy genes, such as ATG5 and ATG7, which promotes cellular survival [151] and parasite infection [152]. Chemical inhibition studies for screening of potential drugs identified GSK2606414 targeting the kinase domain of PERK. It has already been used as an anti-cancer agent [153], and targeting these pathways can have therapeutic implications.

There are significant numbers of secretory proteins produced by the parasite during host invasion. Some of these secretory products are important virulence factors, and processing and transport of these proteins outside the cell [147] comprises a crucial component of parasite life cycle in the host. A number of proteins involved in protein folding and quality control of ER-mediated chaperoning activity involves-calreticulin [154], BiP [155] and protein disulfide isomerase (PDI) [156]. Calreticulin constitutes critical players of ER quality control of secretory proteins. However, its overexpression is associated with decreased survival inside macrophage, and altering the function of ER chaperone [157] affects protein secretion and thus a potential candidate for drug discovery.

ER also remains an important constituent of parasitophorous vacuole (site of parasite residence) as documented by blocking the N-ethylmaleimide-sensitive factor attachment protein receptors (SNAREs) which causes fusion of early secretory vesicles. Blocking SNARE does not affect ER morphology but significantly reduced parasite replication [158] therefore, providing clues for utilizing SNARE complex as therapeutic option for targeting the disease.

On the other hand involvement of protein disulfide isomerase (PDI), a key molecule in regulating ER quality control process of protein processing [159] has also been suggested as one of the demanding drug targets whose depletion is associated with cytotoxic effects and ultimately apoptosis [160, 161]. Chemical inhibition studies has further shed light on the essential role of these chaperones as regulator of parasite survival [160]. Signal peptide peptidase (SPP) is amongst other important candidates, due to its high selectivity for misfolded/unstable proteins for degradation [162], and makes them a choice for drug target. As a pathway for identification of drug targets, the ER mediated pathway of protein processing has not been much explored therefore, there remains a need to study the role of

ER proteins which serves as the key regulators of parasite life cycle. This would be of great help in laying the base for the drug designing and therapeutic intervention.

Epigenetic manipulations as therapeutic option

Parasitic organisms have evolved the strategy to promote their survival in the host by playing with the host transcriptional machinery. Alteration in the host transcriptome and proteome profiles using different weapons of parasite origin leads to disease pathology.

In recent times, epigenetics has gained attention of scientists, where heritable changes in the gene expression does not involve modification of core DNA sequence but depends on alteration of either DNA/histones. This alteration of DNA/histone affects the chromatin structure and gene expression. These modifications in addition to the changes in DNA methylation subverts the host cellular responses and drives the expression of genes favoring the growth and survival of the pathogen. These heritable long term changes promote disease pathogenesis and persistence of the pathogen within the host. In this section we discuss how epigenetic changes are brought about by the parasite, its implications in immune regulation and as a therapeutic target. The changes in DNA methylation attenuated NF κ B1-mediated pro-inflammatory signaling [163], inflammatory responses [164], apoptosis, pathogen induced signalling and changes in host behavior. However, limited evidences are available for epigenetic effects of *Leishmania* infection on host cell, with recent research suggesting for epigenetic reprogramming of macrophage during *L.donovani* infection [165].

Recent years have experienced a plethora of researches focusing on the epigenetic phenomenon in understanding disease biology. Epigenetic analysis provides fast, reversible and ready access to phenotypic changes which shapes the host-parasite interactions. These changes are brought about by the histone modifications, transcriptional and post-translational regulatory circuits by altering the histone-DNA interactions. The remodelling of chromatin by *T.gondii* downregulated TNF- α [166], altered STAT1 mediated IFN- γ responses [167]. Moreover, the host epigenetics has also been known to be altered by microbial secretory products as reported from *Listeria* [168], influenza virus [169] and *Legionella* [170] studies. Diverse effectors from microbial agents have been known to inhibit cellular machinery including MAPK, IFN and transcription factor NF- κ B signaling [171] as reported from *Mycobacterium* [172], influenza virus [169]. Therefore, providing compelling evidences for the pathogen mediated induction of epigenetic modification in the host.

Recently, non-coding RNA (nc RNAs) and miRNAs have also been reported as the regulators of epigenetic processes by DNA silencing by post-transcriptional regulation [173]. Epigenetic control of pathogen virulence has been well known from studies including *Salmonella*, where lack of DNA adenine methyltransferase led to impaired invasion capacities, envelope instability, reduced motility in the pathogen [174]; *Plasmodium* switches its protein expression to overcome host immune responses [175].

In the light of limited data available for the effect of epigenetic changes in the host macrophages and most of the evidences, available till date, are correlational, and pathogenic proteins mediated changes in the host epigenetics are still to be described. The scope for

targetting the parasite-mediated changes in the host epigenome, and directly modulating the host epigenome, arresting the pathogen developmental switches inside host or limiting their virulency with the use of chemical inhibitors, RNAi (RNA interference), gene knockout, etc. could opens up new avenues for targeting histone modifying enzymes, DNA methyltransferases and chromatin therefore, providing the science with the new concept of “epigenetic therapy”, which may serve to deal with the issues related with drug resistance.

Iron homeostasis as therapeutic target

Iron is one of the crucial components of the cell which cycles between ferrous and ferric forms enabling the organisms for performing several life sustaining biological processes. Iron forms active part of diverse proteins such as aconitase, ribonucleotide reductase, cytochromes and Fe-S proteins of electron transport chain [176]. Additionally, it forms an active ingredient of collagen, tyrosine catecholamines [177] as well as immune responses in mammals. Iron homeostasis is stringently regulated in prokaryotes as well as eukaryotes. Leishmania has LIT1 (Leishmania iron transporter) as one of the crucial iron transporters [178], expressed in iron deficient environment and serves to increase iron flux. This serves as a trigger for parasite differentiation. Genetic deletion studies revealed the replication defect inside macrophages and avirulent state of the parasite [179]. Recently, LIT1 up-regulation has been associated with increased super oxide dismutase activity and reactive oxygen species therefore, regulating parasite differentiation [180]. Genome researches have led to the identification of LFR1, membrane protein with heme, FAD and NADPH-binding sites in transmembrane regions. Together with LIT1 it equips Leishmania with inorganic iron acquisition pathway. LFR1 accounts for ferric reductase activity and deletion studies have reported its role in parasite differentiation [181]. These findings suggested for therapeutically targeting these transporters for disease intervention.

Iron is also a constituent of mitochondrial superoxide dismutase which mediates parasite protection and redox signalling as demonstrated by RNAi-studies. Genetic deletion studies further validated their role in driving susceptibility of parasite to ROS-induced stress and differentiation [182]. Thus, this molecule should be considered as drug target. Bioinformatics and florescence microscopy based study revealed LmABCB3 as important mitochondrial target molecule. It is ATP-binding cassette (ABC) half-transporter with metal binding domain responsible for mitochondria dependent heme and iron-sulfur cluster synthesis. Thus, LmABCB3 transporter is essential for parasite survival, and represents novel target for combating leishmaniasis [183].

LABCG2, another ABC subfamily transporter, plays vital role in parasite virulence. It is required for externalization of phosphatidylserine, as an adaptive strategy for macrophage invasion. Mutation studies from this transporter indicated for decreased pathogenesis as well as virulence of the parasite [184]. Moreover, evidences from susceptibility and biotinylation assays reported the overexpression of LABCG2 in antimony resistant isolates as documented by sequestering metal-thiol conjugates [185]. Similarly, LABCG1 was also found as the key player working hand in hand with LABCG2 in regulating metacyclogenesis, infectivity, oxidative stress and autophagy [186]. Thus, these transporters could be used as therapeutic targets, and with these, cure of drug resistance is also a possibility. LHR1 remains another

important target molecule of iron homeostatic pathway of parasite. Fluorescence tagging and genetic deletion studies has unveiled the role of this transporter in parasite survival [187, 188] and virulency [189], targeting this transporter for therapeutic purpose can be effective in these intervention of trypanosomatid diseases.

LMIT1, mitochondrial iron transporter identified in *Leishmania* has been found to be crucial for parasite viability and differentiation. The parasites with reduced LMIT1 expression showed growth defects, severe defects in iron content and susceptibility for ROS. The involvement of this iron-dependent transporter in mitochondrial redox balance and parasite virulence opening horizon for targeting iron metabolism for therapeutic benefit [190].

Other host based iron homeostatic regulators include-DMT-1(Divalent metal transporter) [191], Tf/TfR (transferrin/transferring receptor)[192], hepcidin[193], ferroportin [194] and IL-6 receptor [195] which can be selectively targeted for therapeutic benefits.

Kinome based therapeutic targets

Kinome represents the complete set of protein kinases encoded by the genome. Protein kinases serve as an important arm of cellular programming. Post-genomic era has experienced technological advancements which has served as the landmark in understanding the disease biology and unraveled the relative contribution of parasite genes in disease progression [196, 197]. In-depth genomic and functional analyses have suggested phosphoinositol kinase (PIK) pathway as popular targets for development of drugs in the treatment of several diseases. Recent years have experienced a plethora of chemical and natural products targeting specific protein kinases, several are under clinical trials.

Protein kinases have a crucial role to play, in parasite life cycle as discussed in recent articles including mitogen-activated protein (MAP) kinases, PI3 kinases, NF- κ B signalling [198-201]. On the other hand, complement receptors also play important role in activation of protein kinases in turn regulating host immune responses.

Cyclin-dependent kinases (CDK) are most studied amongst kinases as a target for leishmaniasis. As already discussed above, CDKs play important regulator of cell cycle, therefore, inhibitors targeting the ATP binding to the catalytic site of CDKs [202], could serve as potent drug targets. In this context, flavopiridol is worth mentioning as it is the first CDK inhibitor that reached clinical trial. Recently, Abl-family of kinases (discovered as oncogene in Abelson leukemia virus) have been reported by Wetzel *et al.* as non-receptor kinases[203]. These kinases have been known to play important role in cell-cell contact, cytoskeletal rearrangement during phagocytosis and cell motility [204]. The chemical inhibition studies and knock down studies has further elaborated the role of these kinases during infection. The combination of Imatinib (Abl-family kinase inhibitor) with conventional chemotherapeutic choices could have a potential role in controlling parasite. Abl-family kinases during *Shigella* [205], *Chlamydia* [206] and *Mycobacterium* infection [207] have already been established. MAPKs are signal transducers that regulate cytoskeletal rearrangements, proliferation, differentiation, immune responses and diversity of other cellular responses. The MAPKs displays conserved residues for their regulatory roles. The

cascade of events initiates with MAPK activation, followed by its phosphorylation and involvement of different transcription factors that alters gene expression [208] and epigenetic modifications by affecting histone remodeling [209], JNK(c-Jun activated kinases) [210] and p38 stress-response MAPKs [211]. Although modest body of work has been reported, however, utilizing the phylogenetic differences between MAPKs of parasites and host as well as the upstream MAPK regulators can be of great relevance in developing new therapeutic options. LmaMPK7 is the only MAPK known for controlling the parasite virulence, in its active form [212] while decreased LdMAPK1 has been associated with antimony resistance [213]. Therefore, these studies lay the foundation stone for exploiting this MAPK for development of the noninfective parasite which can be of relevance as an immune boosting strategy for controlling parasite. Protein kinase regulated by RNA constitutes another class of kinases involved in promoting parasite burden during *L. amazonensis* infection [214] Similar studies from Mycobacterium infection [215] suggest designing of inhibitor based approaches for targeting this protein kinase towards therapeutic advantage. However, reports from *L. major* infection were contrasting with the *L. amazonensis* dataset [216], and this could be attributed to different host-parasite interaction and downstream signaling. Other kinases include casein kinase which remains important drug target due to its essence for parasite survival and virulence [217, 218]. NEK kinases [219, 220], CLK and DYRK are other protein kinases [221] are potential candidates as drug targets. Several CDK family members including cdc2-related kinases(CRKs) [222], tyrosine kinase[223], glycogen synthase kinase [224], PI3 kinase, Src kinases [225], JNK [226] are other potential drug target.

Calcium homeostasis as therapeutic target

Calcium remains an important component of cellular homeostatic machinery essential for cell viability for all organisms ranging from mammals to parasites and non-mammals [227, 228]. Any kind of perturbation in the calcium homeostasis severely affects the cell viability, leading to cell death [120, 229]. Calcium also plays crucial role in protozoan parasites by regulating flagellar, ciliary movements [230], exocytosis and regulator of several enzymatic activities including adenylate cyclase [231], cAMP phosphodiesterase [232], protein kinases[233] and guanylate cyclase [234].

In trpanosomatids, the levels of calcium regulated at cytoplasmic level where the regulatory control is under: ER, mitochondria and acidocalcisome[235]. At the plasma membrane level, Ca^{+2} -ATPase, membrane channel is regulated by calmodulin in trypanosomatids [236, 237], another transporter in internal membrane of mitochondria drives calcium-ion accumulation [236, 238]. All these system of homeostatic machinery work in coordination for maintaining the intracellular calcium equilibrium to serve as signalling messenger[227]. Calcium has also been known to play crucial role in differentiation of the parasite and its thermotolerance [239-241].

The alteration in the calcium levels are sensed by calcineurin which serves to activate the signaling cascade required for parasite differentiation and adaptation to cellular stress. However, genetic deletion of calcineurin led to devastating effects including changes in membrane fluidity, unfolded protein response along with the loss of virulence [242].

ER remains the largest storage reservoir of calcium with different calcium influx and efflux pathways. Calreticulin is calcium storage protein in ER [232], SERCA (Sarcoplasmic reticulum Ca⁺²-ATPase) has been demonstrated in different trypanosomatids which serves as a virulence factor in *Leishmania* [243] could act as a potential therapeutic target. Calcium sensitive protein aequorin in the nuclear membrane has been known to mediate the flux of calcium in the nucleus of trypanosomatids[244]. Also, the nuclear membrane shows the presence of proteins that stain for BiP, calreticulin and TcSCA [245]. Mitochondria possesses calcium uniporter as already mentioned above, also, literature has evidences for calcium-uptake and release channels in trypanosomatids [238, 246, 247].

Acidocalcisome constitutes another important target site for the storage of calcium, pyrophosphates, polyphosphates and other elements [235, 248]. It has PMCA-type Ca⁺²-ATPase responsible for calcium influx [235], while Ca⁺²/H⁺ exchanger has been demonstrated to be responsible for calcium efflux [249]. Calcium ionophores play important role in host-parasite interaction as demonstrated in *T.cruzi* infection, regulation of several protein kinases[221, 250] and parasite differentiation [251].

Noteworthy role of calcium in regulating parasite life cycle emphasizes for targeting calcium transporters could have therapeutic implications. In this regard, calcium channel blockers-fendiline, mibefradil and lidoflazine, etc. were found to have potent antileishmanial activities. However, the combination of these calcium blockers with the conventional chemotherapeutic options further elicited additive effect for clearing off the parasite by inducing mitochondrial depolarization, increased reactive oxygen species generation without plasma membrane disruption [252]. Therefore, use of calcium channel blockers has suggested for their utility as therapeutic candidates alone or in combination with chemotherapy for treating leishmaniasis. Likewise, bepridil has shown good efficacy[253], verapamil in combination with meglumine antimoniate has synergistic effect on parasite clearance[254].

Mitochondria remain the site for electron transport chain where four complexes coordinating the entire process. Rotenone-insensitive, NADH: quinone oxidoreductase constitutes the complex I in *Leishmania* and *Trypanosoma* without any human counterpart[255, 256]. The reports from various researches have suggested for the presence of complex II, and limited involvement of electron transport between complex I-III, makes succinate, primary electron donor for energy production. It has been characterised in *Leishmania* [257] and *Trypanosoma* [258], its absence in mammalian mitochondria makes it potential candidate for the drug designing.

Early efforts to target ETC dated back to the 90s when complex III (cytochrome bc₁ complex) was analysed and amino acid differences in ubiquinone binding with the human counterpart made it an interesting target for drug development [259]. Complex IV has subunits encoded by nuclear and mitochondrial DNA [260], present in both *Leishmania* (Ldp27) [261] and *Trypanosoma* (Tb11.0400) [262], correlated with infectiousness of the parasite. Trypanosomatid ETC involves alternative oxidase as terminal electron acceptor in ETC, lost during evolution in mammals[263], does not participate in generating proton motive force across mitochondrial membrane [264].

Other therapeutic targets

Leishmania undergoes preadaptation to the environmental conditions of the host macrophage. Autophagy constitutes an important component of parasite life cycle which involves cathepsins and cysteine peptidases for controlling protein turnover crucial step in parasite development and differentiation [265]. This established these molecules as therapeutic target for drug development.

Immunotherapy is the hottest area of research for disease intervention these days. It involves the host-directed immunotherapies, that targets the immune and inflammatory pathways for alleviating disease pathology [266]; cytokine based immunoregulatory therapy [267]; cell based therapy using dendritic cells, mesenchymal cell, etc. [268-271] and immune checkpoint blockade as well as monoclonal antibody based immunotherapy [272-274]. Combination therapy with monoclonal antibody has shown promising results in clearing off parasite burden even at a suboptimal dose of conventional chemotherapeutic approach [272]. Indeed, immunotherapy is in itself a broad research area which is beyond the scope of this article.

Recently, nicotinamidase, enzyme involved in assimilation of nicotinamide, nicotinic acid, nicotinamide riboside to synthesize NAD⁺ by salvage pathway. It constitutes a key component of parasite development and virulence, add-back and mutational studies has attracted the scientific community for designing novel inhibitors and utilize this molecule as a therapeutic target [275]. *Leishmania* utilizes purine pathway from mammalian system using different nucleoside transporters. Phosphoribosyltransferases constitutes a key component of purine salvage pathway including three main enzymes: hypoxanthine-guanine phosphoribosyltransferase, xanthine phosphoribosyltransferase and adenine phosphoribosyltransferase [276, 277]. *Leishmania* has several enzymes for breakdown of host nucleosides, nucleotide and nucleic acid before incorporation into its purine pools. Cloning, expression and immunolocalization has led to characterization of several nucleoside hydrolases [278-280], several nucleases/nucleotidases that generates free nucleosides [281] thereby making the purine accessible for translocation through transporters into the parasite.

Several transporters designated for purine acquisition include: LdNT1 [285], LdNT2 [283, 284], LdNT3 and LdNT4 [285]. Nucleobase/proton symporters transporters from *L. major* – LmaNT3 and LmaNT4 [286], demonstrated for their important role in parasite viability inside macrophage therefore, providing clues for the necessity of targeting the salvage pathway and acquisition systems for therapeutic purpose.

Glycoconjugates, present another interesting target molecule for therapeutic intervention as they are the key player in parasite survival and infectivity. Lipophosphoglycan (LPG) and proteophosphoglycan (PPG) are phosphoglycans with host immune subversion properties [287, 288], genetic deletion and mutation studies [289] established their importance as therapeutic target. Therefore, the functional role of these glycosylphosphatidylinositol (GPI) molecules and their biosynthetic pathways served as an essential factor for parasite virulence

[290] opening avenues for their exploitation for drug designing and discovery. Tubulins are another target molecule of interest for therapeutic intervention [291].

DNA topoisomerases plays vital role in DNA replication, repair, transcription and recombination forms another candidate for drug discovery. It has gained attention in recent years after success of camptothecin for cancer treatment when camptothecin analogs – topotecan, gimatecan and irinotecan were shown to exhibit anti-leishmanial activity [292].

Recent study by Abidin *et al.* reports the activation of hematopoietic stem cells by the parasite, which in turn promotes infection. These finding suggest hematopoiesis as a potential therapeutic target in leishmaniasis [293].

Apoptotic pathway serves as regulator of multitude of physiological processes ranging from cell division to cell death. Several proteases, metacaspases and chemical inhibitors have been proven to possess anti-leishmanial activity [294-296], also they play roles in determinant of host-parasite interaction as well as disease pathogenesis[297]. Therefore, the apoptotic pathway can serve as interesting therapeutic target for drug development.

Multiscale mathematical modeling and simulation have also led to the discovery of IPC synthase, a sphingolipid synthase present in acidic macrophage phagolysosome. It forms an essential component of disease pathogenesis and serves as another vital candidate for drug discovery therefore, interactome analysis can serve for the drug designing [298].

Cholesterol forms another important therapeutic target for leishmaniasis as documented by cyclodextrin-based alterations in membrane cholesterol levels [299]. Sterol 14 α -demethylase also forms an important therapeutic target for antiparasitic chemotherapy [300].

Autophagy associated peptidases also constitutes the class of therapeutic targets involved in protein turnover and remodelling, therefore constituting an important component of development, differentiation and virulence of parasites[301].

The parasite derived toll-like receptors (TLRs) ligands also forms target for therapeutics and vaccine development. Several membrane protein transporters, glucose transporters, sugar-nucleotide and purine transporters [302] are also potential targets for drug development. The list of candidate molecules does not end here there are many more under evaluation and others with unknown functions have yet to be explored.

Expert opinion and Conclusion

In this review we have analyzed the recent developments in search for the candidate molecules for their potential use as therapeutic targets. The drawback associated with current chemotherapeutic approach led to the introduction of the single dose liposomal amphotericin B which showed satisfying treatment outcomes. This was the major breakthrough in drug development as to overcome the issues related to the development of drug resistance because of suboptimal drug concentrations and patient non-compliance. Additionally, combination therapies [303] have been expected to be efficacious in preventing disease recrudescence and provide better patient compliance. It has paved the way for

targetting multiple pathways in the parasite by inducing synergistic effect for clearing off the infection. However, the identification of combination regimen with characteristic features of good tolerance, minimal need of clinical observation and ambulatory care will be the priority for drug development. Therefore, discovery and development of new therapeutic tools remain highest priority.

The technical advances in nanotechnology and system biology have contributed to development of the drug designing and delivery systems with fairly good treatment outcomes in different models. Plant products and chemical compounds have been raising flags as potential and inexpensive alternatives for different forms of leishmaniasis which has been expected to consolidate the needs of low-income patients. Similarly, immunomodulatory and host-directed immunotherapies have further expanded the horizon for the multifaceted approach for disease cure. It appears that systematic molecular and cell biology approaches, drug repurposing, screening of off-the-shelf drugs have served for identification of novel candidates for drug discovery. Last decade has experienced boom in the immunotherapy and immunochemotherapy defines the remarkable progress in the research for therapeutics. However, variability in the potency of host responses to different pathogens needs to be standardized which calls for heavy investments from government and industries. The search for new therapeutic targets continued and led to the study of metabolic pathway as drug targets for therapeutic benefit. There are several pathways as the targets for the development of therapeutic tool several of which have being documented for their ability to improve clinical symptoms and decrease parasite load. Mitochondrial targeted approaches have significantly gained the interest of the scientific communities however, there are many other potential pathways that have emerged in recent years. Therefore, there always remains an urgent need for the development of therapeutic option with potential of cure or reverse severity of clinical manifestation. Leishmaniasis elimination program in developing nations has got support from international community for ensured treatment of disease has been the helping hands for the local authorities for drafting the roadmaps for combating the disease. This has led to steep decline in the number of cases reported per year, however, the achievement of elimination goal still requires efforts at ground level for following up the past cases to monitor the proper clinical cure as well as prevention of disease transmission. In order to bolster the drug development, identification of new targets can contribute significantly towards strengthening the pipeline for disease elimination. This is expected to reap rewards in the form of leishmaniasis free nations in coming years.

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**Considerable interest

* Special interest

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Highlights

- The conventional chemotherapy approaches are associated with toxic side effects which called for the development of alternative approaches.
- The technical advancement in the post-genomic era has explored many new drug targets.
- The advances in metabolomics, system biology and nanotechnology approaches has led to better understanding of parasite biology therefore, better chances for identification of drug targets and their therapeutic potential.

Research findings targeting metabolic pathways in *Leishmania*

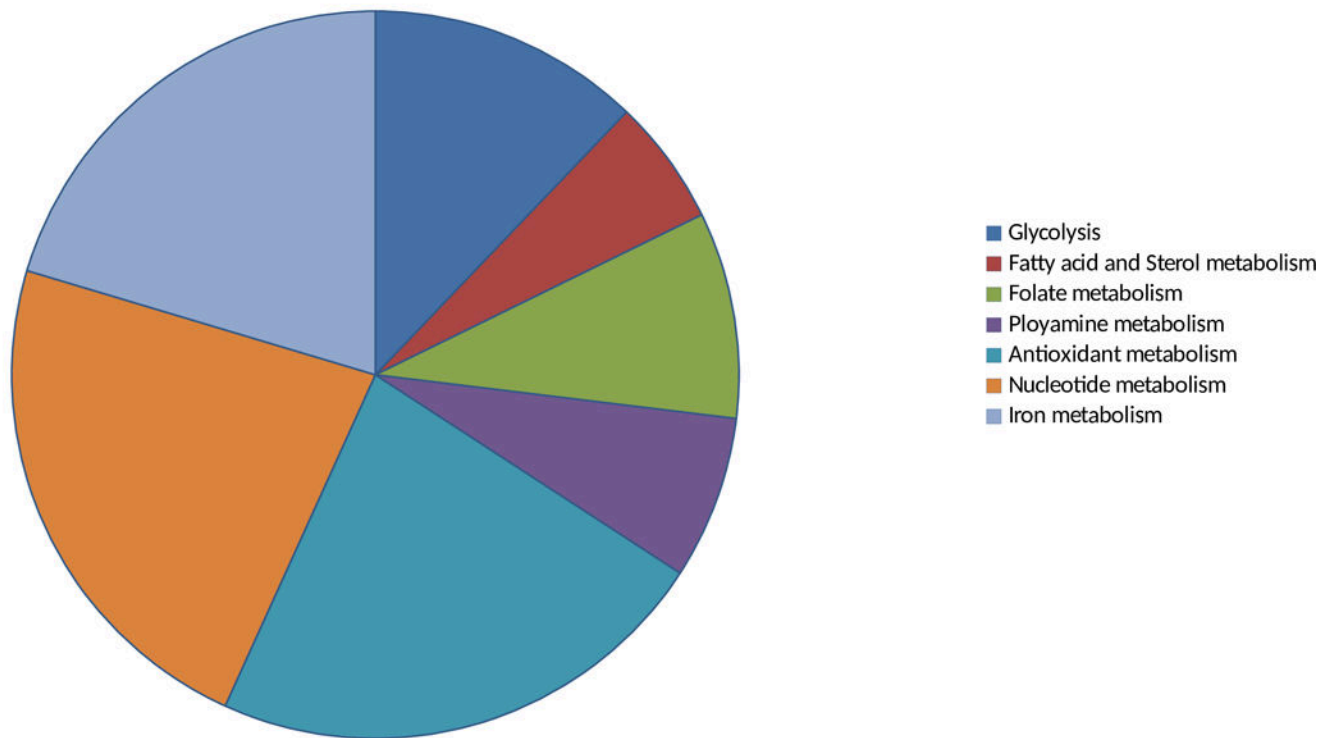


Figure 1. Overview of different metabolic pathways studied in last five decades in *Leishmania*. The pie chart represents different researches in the field of *Leishmania* throughout the world targeting different metabolic pathways.

Table 1

Anti-leishmanial activities of some plant products/compounds.

Plant products/ compounds	Standard Drug	Plant	Species	References
Oxylipin (Polyacetylene)	Oxylipin (3S)-16, 17-didehydrofalcariinol	<i>Tridax procumbens</i>	<i>L. mexicana</i>	[304]
Flavonoids	Luteolin	<i>Vitex negundo</i>	<i>L. donovani</i>	[305]
	Quercetin	<i>Fagopyrum esculentum</i>	<i>L. donovani</i>	[306]
	Quercetin and its derivatives	<i>Kalanchoe pinnata</i>	<i>L. amazonensis</i>	[307]
	Quercetin	<i>Kalanchoe pinnata</i>	<i>L. chagasi</i>	[308]
	Ethanolic extract	<i>Piper betle L.</i>	<i>L. donovani</i>	[309]
	Methanolic extract	<i>Piper betle L.</i>	<i>L. donovani</i>	[310]
	2''-acetylpetolaroside	<i>Delphinium staphisagria</i>	<i>L. infantum</i> and <i>L. braziliensis</i>	[311]
	Guaiacolide	Tanacetum parthenium (L.) Schultz Bip	<i>L. amazonensis</i>	[312]
	Quercetin-3-O-methyl ether	<i>Strychnos pseudoquina</i>	<i>L. amazonensis</i>	[313]
	Plant extract	<i>Bidens pilosa L. and Punica granatum L.</i>	<i>L. amazonensis</i>	[314]
Alkaloids	N-Methyltetrahydroberberinium iodide	<i>Enantia chlorantha</i>	<i>L. donovani</i>	[315]
	Ancistrocalaine A	Ancistrocladus ealaensis	<i>L. donovani</i>	[316]
	Quinoline alkaloid	Galipea longiflora	<i>L. amazonensis</i>	[317]
	2-n-Propylquinoline		<i>L. amazonensis</i>	[318]
	Julocrotine, a glutarimide alkaloid	<i>Croton pullei var. Glabrio</i>	<i>L. amazonensis</i>	[319]
	Methanol extract	<i>Nuphar lutea</i>	<i>L. major</i>	[320]
	Quinoline alkaloid	<i>Galipea longiflora</i>	<i>L. amazonensis</i>	[321]
	Ethanolic extract	<i>Senna Spectabilis</i>	<i>L. major</i>	[322]
	Bisbenzylisoquinoline	<i>Cissampelos sympodioides Eichl.</i>	<i>L. chagasi</i>	[323]
	Furoquinoline alkaloids	<i>Helietta apiculata</i>	<i>L. amazonensis</i>	[324]
	Securinega alkaloids	<i>Margaritaria nobilis</i>	<i>L. amazonensis</i>	[325]
	Dicentrinone	<i>Duguetia furtifera</i>		[326]
Triterpenoids	Triterpenoid extract	<i>Trichosanthes dioica</i>	<i>L. donovani</i>	[327]
	Sesquiterpene lactone-rich dicholomethane fraction	<i>Tanacetum parthenium</i>	<i>L. amazonensis</i>	[328]
	Oleanolic- and ursolic-containing triterpenic fraction	<i>Baccharis uncinella</i>	<i>L. amazonensis</i> and <i>L. braziliensis</i>	[329]
	Taxodione	<i>Salvia deserta</i>	<i>L. donovani</i>	[330]

Plant products/ compounds	Standard Drug	Plant	Species	References
	Artemisinin	Artemisia annua	<i>L. major</i>	[331]
	Abietane diterpenoids	<i>Taxodium distichum</i>	<i>L. donovani</i> and <i>L. amazonensis</i>	[332]
	trans-dihydrocrotonin	<i>Croton cajucara Benth.</i>	<i>L. amazonensis</i>	[333]
Chalcones	Licochalcone A	Chinese liquorice	<i>L. major</i>	[334]
	20 naturally occurring chalcones	Piper aduncum	<i>L. donovani</i>	[335]
	Adunchalcone	Piper aduncum L.	<i>L. donovani</i>	[336]
	Dihydrochalcones	<i>Piper elongatum</i>	<i>L. amazonensis, L. braziliensis and L. chagasi</i>	[337]
Quinones	Plumbagin	<i>Piper elongatum</i>	<i>L. braziliensis</i>	[338]
	3,3'-Biplumbagin	<i>Pera benensis</i>	<i>L. amazonensis</i>	[339, 340]
	1-Acetylbenzochromanquinone	Cephaelis camponutans	<i>L. donovani</i>	[341]
	N-Methyliriodendronine	Stephania dinklagei	<i>L. donovani</i>	[342]
	2-methyl-5-(3'-methyl-but-2'-enyl-oxo)-[1,4]naphthoquinone (1)	Plumbago zeylanica	<i>L. donovani</i>	[343]
	Methanolic extract	Nigella sativa L	<i>L. tropica</i> and <i>L. infantum</i>	[344]
Saponin	Hederagenin	Ivy Hedera helix	<i>L. tropica</i> and <i>L. infantum</i>	[345]
	α -Hederin	Hedera colchica	<i>L. mexicana</i>	[346]
	β -Hederin	<i>Maesa balansae</i>	<i>L. infantum</i>	[347]
	Hederacolchiside A1	<i>Maesa lanceolata</i>	<i>L. infantum</i>	[348]
	Maesasaponin V.3 and VI.2	<i>Maesa balansae</i>	<i>L. infantum</i>	[349]
	Maesabalides III	<i>Haplophyllum bucharicum</i>	<i>L. infantum</i>	[350]
	Maesabalides IV	<i>Propolis</i>	<i>L. amazonensis</i>	[351]
Lignan	Diphyllin	<i>Propolis</i>	<i>L. amazonensis</i>	[352]
4-Aminoquinoline	Neolignan 2,3-dihydrobenzofuran AMQ-j		<i>L. infantum</i>	[353]
N-cinnamoylated aminoquinoline			<i>L. amazonensis</i>	[354]
7-chloro 4- quinolinylhydraz one			<i>L. amazonensis s.L. braziliensis s.L. infantum</i>	[355]
8-hydroxyquinoline				
1,2,3-triazole salts (Biososteres)				

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Plant products/ compounds	Standard Drug	Plant	Species	References
N-benzyl-1-Hbenzimidazole			<i>L. amazonensis</i> <i>L. mexicana</i> , <i>L. donovani</i> , <i>L. braziliensis</i>	[356]