INTRODUCTION

Leishmaniasis is a global vector-borne nuisance of the poverty-striken developing countries with unsatisfactory socioeconomic sphere (Neris et al., 2013; Oryan et al., 2014; Lemma et al., 2017). It is a widespread perilous cause of mortality in the tropical and subtropical countries which imposes real challenge in the progress and development of humankind (Daneshbod et al., 2011; de Medeiros et al., 2011; Monzote, 2011; WHO, 2017a). It is an opportunistic disease caused by a parasitic protozoan of genus Leishmania which has been found to have evolved approximately 90-100 million years ago (Harkins et al., 2016). Out of 53 known species of this genus, about 20 are significant to the humans (Barratt et al., 2017). Leishmaniasis is a sandfly borne communicable disease as it is broadcasted by different species of anthropophilic phlebotomine female sandfly vector (Moriconi et al., 2017). The sandfly of genus Phlebotomus and Lutzomyia are the known vectors of Leishmania in the Old World and New World respectively (Akhoundi et al., 2016; Souza et al., 2017).

More than one-sixth of the total populace of world i.e. approximately a billion inhabitants are suffering from the NTDs. Leishmaniasis is also entitled as one of most neglected disease by the 2020 roadmap of WHO and CDC. The Global Burden of Disease Study has declared leishmaniasis to conclude in 1356.46 thousand years of disability-adjusted life years (DALYs) (CDC, 2017; Mitra and Mawson, 2017). According to the epidemiological data more than 616 million people are at the peril to strike the infection in about 97 countries which are primeval to the leishmaniasis. Moreover, based on the global estimation studies about 300,000 disease incidences with 20,000 deaths eventuate every year due to leishmaniasis (NVBCDP, 2017).

In recent years the outspread of leishmaniasis has intensified due to climate changes, ecological disasters, destitution, conflicts and population movement or migration as all these activities have resulted in the expansion of the vector. Additionally the spread of zoonotic reservoir hosts like dogs which convey cardinal role in the transmission cycle is also a decreed reason for increasing outbreaks of this infectious disease (Khezzani and Bouchem, 2017; Moriconi et al., 2017; Morillas-Márquez et al., 2017). In agreement to all these mentioned factors epidemiology of leishmaniasis keeps fluctuating and remains inconsistent (Steverding, 2017).
The clinical aftermath of leishmaniasis ranges from finite dermal or grossly affected mucous membranes to potentially fatal systemic manifestation. The paradigm of disease reckons on species of infecting parasite and on the state of immune protective system of the host (Scorza et al., 2017).

Cutaneous leishmaniasis (CL) is the most frequent form of leishmaniasis throughout the world which ensues with painless lesions on skin at the site of bite of sandfly (Aronson et al., 2017). It is principally caused by L. tropica, and sporadically by L. infantum and L. donovani complex species (Khatri et al., 2016). According to the WHO reports from the year 2005 to 2015 the burden of CL has increased by about 2 million new cases (WHO, 2017b). It is interpreted that the highest burden of CL is chiefly shared by the countries of Africa and the Middle East with both male and female at similar probability of incurring it (Karimkhani et al., 2016). The cases of this disease has hiked owning to altering environment and rising disputes as in many war zones of Afghanistan, Syrian Arab Republic and Iraq (Du et al., 2016). In Iran CL is considered to be the foremost zoonotic disease propagated by the vectors (Khosravani et al., 2016). In India, CL prevails in northern parts involving some pacthes of Rajasthan mainly Thar Desert, in Punjab, Himachal Pradesh, Delhi, Uttar Pradesh and Kerala (Aara et al., 2013; Kaul et al., 2016; Sinha et al., 2016). No prime efforts have been taken for the management of this disease as it is not life menacing. The signs and symptoms of CL start appearing after 2 to 8 weeks of the sandfly bite. An active CL is characterized by formation of lesions which evolves from papules and ultimately leads to scaly and crusted ulcers on the skin. The most exposed parts viz. forehead, face and neck are the frequent regions which develop lesions (Aoun and Bouratbine, 2014; Rather and Yaseen, 2017). The CL may involve lesion on trunk region which can spread to a massive area with eroded and crusted presentation which may also encompass exudation of pus. The number of different cells like histiocytes in the connective tissue, neutrophils, lymphocytes and plasma B cells show numerous fluctuations during CL (Patel, 2017). The formation of ulcers and tissue necrosis in CL is a consequence of increased proliferation of NK cells, CD3⁺CD4⁻CD8⁻ T lymphocytes and CD4⁺ T cells (Campos et al., 2017; Ferraz et al., 2017). CL infection impairs microbiota of skin not only in the vicinity of the lesion but to the distant regions which further aggravates the disease (Gimblet et al., 2017). Although these lesions are self-mitigating but they demise by persisting scars which leads to social
Introduction

stigma (Karimkhani et al., 2016; Al-Kamel, 2017). In many instances in CL relapses occur and culminate in another austere tuberculosis mimicking form i.e. leishmaniasis recidivans (LR) which is a chronic condition. In this unusual form of CL the lesions extend from the dermal regions to the mucosal membranes of the mouth and nose (Dassoni et al., 2017).

The CL is often underestimated and uncorroborated with other clinical conditions involving various dermal infections like leprosy, impetigo, lupus vulgaris which procrastinate the diagnosis and contribute to more disseminated disorder (Merino-Espinosa et al., 2017). Biopsy of the lesion region, staining of the tissue smear, culture in NNN and PCR are the few diagnostic assays for CL (Downing and Tyring, 2016; Patel, 2017).

MCL or espundia results in granulated abnormal plaques and purulent pustules on nose, lips, cheeks, palate and mucosa of oral cavity with concomitant destruction of nasal septum and its supporting tissue, columella. In severe manifestations it may grow and infect pharynx and larynx of the individual which happens to be fatal (Ekiz et al., 2017). It may occur as the extended episode of CL or even independently by L. amazonensis, L. (Viannia) braziliensis, L. (V.) guyanensis and L. (V.) panamensis (Shirian et al., 2012a, 2012b; Cincurá et al., 2017). The cases of MCL have mainly been encountered in America involving vectors, Lutzomyia intermedia and L. whitmani (Gomes et al., 2017). Although the chances of instigating MCL are rare, only about 3% of it is highly direful disorder leading to exorbitant disfigurement. The incidence of MCL is greater in old people and it becomes more severe in instances of delayed diagnosis (Cincurá et al., 2017).

VL or kala-azar is a systemic form of leishmaniasis disseminating in the entire reticuloendothelial system with highest likelihood of mortality. It is pervasive in many nations encompassing Africa mainly, Sudan, South Sudan and Ethiopia, sections of America mainly Brazil and in India and its neighboring countries, Bangladesh, Nepal, and Pakistan (WHO, 2016; Idris et al., 2017). Total of 54 districts of India sustain the burden of VL, among which 33 are of Bihar, 11 of West Bengal, 6 of Uttar Pradesh and 4 of Jharkhand. Besides this, many sporadic cases have also been recorded from non-endemic areas of Madhya Pradesh, Himachal Pradesh, Assam, Sikkim, Kerala and Uttarakhand (NVBDCP, 2017). Greater than 70% instances of VL are recorded from an impoverished state, Bihar (Das et al., 2016). Symptomatic VL is an appalling
disease with multifaceted non-specific clinical presentations like high degree of pallor (Kumar Bhat et al., 2017), enlargement of liver (Hammami et al., 2017) and spleen, hypercellular bone marrow, inguinal lymph nodules (Clement and Li, 2017), hypergammaglobulinemia (Omachi et al., 2016) and even brain inflammation (Melo et al., 2017). The embellishment of this disease relies on the infecting agent and state of immune system of the host including the fettle of liver, spleen, and number of circulating cells like leukocytes and lymphocytes during and after the treatment (Pedrosa, 2017). During VL increased inflammatory chemokines, undermined expression of receptors of various other chemokines and mitigated levels of monocytes, neutrophils and T cells result in disease ferocity (Singh and Sundar, 2017). L. donovani manipulates the hematopoietic stem cells of bone marrow in an alternative direction in which these cells proliferate and induce the production of diversified immune cells which are highly liberal to the disease thus adjunct the infection (Abidin et al., 2017). Surge in proportion of T-regulatory cells presenting Foxp3+ and IL10Foxp3+ owing to TGF-β plays paramount role in elaborating the parasite load in the bone marrow. On vanquishing these cells the generations of unfavorable IL-10 get reduced and those of protective effector T cells which are known to trigger IFN-γ get supplemented (Kumar et al., 2017). In VL patients neutrophils exhibit imperfect response as it fails to generate ROS and to perform phagocytosis which plays salient contribution in progression of disease (Yizengaw et al., 2016).

The severity of VL is allied with factors like age, sex, nutrition and most importantly immune status (Zacarias et al., 2017). The milieu of VL has intensified in countries like Sudan and Ethiopia due to the expansion of HIV infection as latter makes person immune-compromised which fabricate them as an easy target of VL (Diro et al., 2014b). In the VL-HIV co-infections far more grievous condition has been encountered in terms of hepatosplenomegaly, recurrence and death rate (Leite de Sousa-Gomes et al., 2017).

Post kala-azar dermal leishmaniasis (PKDL) is an indisposition fostered after the episode of VL. It has been documented that about 50% and 10% of the VL cases get evolved to the PKDL in Sudan and Indian subcontinent respectively (Zijlstra et al., 2003; Rahman et al., 2017). Studies indicate that PKDL can result after insufficient treatment with SSG, paromomycin, miltefosine, liposomal amphotericin
B and even in the individuals with no prior VL contraction (Islam et al., 2013). It involves lesions ranging from macules, nodules to the plaques extending from mouth to trunk region, even involving the mucosal membranes (Ramesh et al., 2015). In PKDL intra-oral pathologies involving vestibule, mucosa and palate can also occur (Misra et al., 2017). After PKDL, chances of developing keratitis in which the patients develop dense corneal infiltrates may ensue (Pradhan et al., 2017). However PKDL never involves spleen and liver, the key affected organs in VL (Gasim et al., 2000). In PKDL the response of cell mediated immunity occurs in accordance with the acuteness or chronicity of the disease. In acute infection CMI is more intense than in the chronic manifestation (Mukhopadhyay et al., 2012; Datta et al., 2015). The circulating levels of IL-10 expressing T cells and CD8+ T cells have been found to increase in the areas of lesions (Ganguly et al., 2010a). It is highly crucial to treat the lesions in case of Indian PKDL than in Sudan as in latter they are self-curing in nature. The failure to treat PKDL may lead to severe complications and it also hinders the avenue of eradication of leishmaniasis (Trivedi et al., 2017).

Many parasites show intracellular manifestations to avoid immune responses of the host (Liehl et al., 2015); one such parasite is *Leishmania*. It shows an obligatory relation with the macrophages of the host (Podinovskaia and Descoteaux, 2015). Parasites mainly inhabit macrophages but it may also be present in other types of cells like monocytes, dendritic cells and neutrophils (Carlsen et al., 2015). *L. donovani* is a dixenous parasite as it requires two different hosts in its life cycle. In each host it displays morphologically as well as physiologically distinct form, amastigote and promastigote. The intracellular amastigotes are minute non-motile spherical forms found in the vertebrate hosts including humans and promastigotes are the free swimming forms found in the gut of sandfly insect vector (Wheeler et al., 2016; Sadlova et al., 2017). Infected sandfly produces promastigote secretory gel which not only manipulates the feeding habits of the insect urging it to feed frequently to the multifarious hosts but woefully this secretory gel also enhances the feasibility of development of the infection in host after the act of biting (Courtenay et al., 2017). When an infected sandfly bites a mammalian host, the promastigotes get endocytosed in the macrophages and reside in the form of parasitophorous vacuole which gets housed in the lysosome forming phagolysosome. Macrophages are known for their remarkable role in phagocytosis and destruction of harmful bodies but in case of
amastigote infection its function gets dwindled. The parasite endures the homicidal essence of macrophages by many ways such as by expressing tryparedoxin peroxidases enzymes which protect them from oxidative stress (Das et al., 2017). Inside the phagolysosome it gets transformed to amastigote form which then undergo mitotic divisions and ultimately result in infecting the whole reticuloendothelial system. It finally leads to collapse of organs and demise of the patient in the omission of relevant treatment (Roberts et al., 2009).

The diagnosis of leishmaniasis should be unambiguous as awry diagnosis retards the correct treatment and transcend the disease to a perilous state. A skilled diagnostic assay should be sensitive, economic, quick, easily accessible and very importantly non-invasive. One of the features of control programme of leishmaniasis is accurate and early diagnosis as it is the mainstay to choose correct therapy. Therefore the branch of diagnosis should prosper in terms of methods and competent markers (Stauch et al., 2014; Akhoundi et al., 2017). Standard methods to diagnose the infection involve direct microscopic recognition of the parasite in the sample of patient like skin, liver, spleen, bone marrow and cultivation of parasite using the vitiated tissue. However, these methods always carry threat of fostering secondary infections to the patient and moreover it requires trained personnel (Akhoundi et al., 2013; Taslimi et al., 2017). Therefore different serological practices are recommended viz. Indirect Fluorescent Antibody Test (IFAT), Direct Agglutination Test (DAT), Enzyme Linked Immunosorbent Assay (ELISA) and rK39 rapid test (Dawit et al., 2013; Lindoso et al., 2016). The success related to specificity and sensitivity of these tests is determined by the antigen used, levels of antibodies and on the status of the host. However in many instances of cured individuals it provides false positive results due to lingering antileishmanial antibodies in the system or negative results in case of infected patients due to low levels of antibodies as in the immunocompromised patients (Akhoundi et al., 2017). Although rK39 immunochromatographic rapid test is a cost effective test requiring no trained personnel, its sensitivity as well as specificity has subsided the already demanding refinement for better results (Kiros and Regassa, 2017; Varani et al., 2017).

Advanced molecular approach such as Polymerase Chain Reaction (PCR) which is a molecular amplification assay is more sensitive than these conventional detection methods. qPCR is a foremost technique for the quantitative detection of
parasite in prevalent as well as relapsed VL cases and in PKDL patients. In comparison to other diagnostic assays this method also allows an easy assessment of the performance of the therapy (Hossain et al., 2017). Among the various PCR methods, SYBR green based qPCR is most specific with superior performance (Gomes et al., 2017). In these molecular assays specific biological molecules possessed by the species of *Leishmania* are used for diagnosis. This may involve nuclear DNA and kinetoplastid marker targets like small sub-unit rRNA gene, internal transcribed spacer 1 (ITS-1) and kinetoplastid DNA (kDNA) (Albuquerque et al., 2017). In canine VL, PQ10 and PQ20 proteins display better index of diagnosis than conventional ITS-1 via ELISA as well as via PCR as these proteins can be detected at very initial stages of the disease and do not show any cross reactivity with other pathogens (Faria et al., 2017).

Tape strip disc is a sampling technique for CL in which the strip is pressed against the tissue gently and then the material collected is examined by PCR. It is an easy and painless technique which can be helpful in field studies as well as in remote areas (Taslimi et al., 2017). In case of PKDL scarcity of the parasites in the lesion makes its diagnosis an intricate challenge. So, xenodiagnosis is employed along with qPCR to confirm the positive patients but it is cumbersome in terms of required expertise and rearing of flies (Molina et al., 2017).

The fight against leishmaniases is perplexing and ill-fated in reference to its zoonotic nature, strong affiliation to vector and also due to its neglected tropical disease status (Molyneux et al., 2017). Moreover, there is insufficient understanding of the processes behind the existence of asymptomatic cases (Andrade-Narvaez et al., 2016). The individuals who remain asymptomatic for the leishmaniasis are at highest risk of spreading disease as the symptomatic patients are rapidly withdrawn from the society for the treatment leaving the untreated asymptomatic. These patients with no symptoms and signs retain parasites in them and act as a main factor in anthropoontic dissemination of this disease (Miller et al., 2014).

The prevention of leishmaniiasis depends on the control of vector by practicing insecticides as well by deterring contact with these vectors (Sevá et al., 2017). Further, the fight against this disease can be won by completely eliminating the reservoirs, by refining the hygienic conditions and by the discovery of cost effective drug against this deadly parasite (Khezzani and Bouchem, 2017). The treatment is
best standard disease limiting strategy. However, the choice of drug, regimen and its dosage is complicated due to the presence of different clinical forms of disease and due to the different reaction of disparate patients. Thus the therapeutic approach should be subjective according to the parasite and host with special attention to the immunocompromised patients. Varied options of parenteral, topical and oral medications are available in the market for the treatment of this disease (Blum et al., 2014; Sundar and Singh, 2017).

The antimony-derived compounds are the basic treatment options adopted in the developing countries (Légaré and Ouellette, 2017). The pentavalent antimonials include sodium stibogluconate (SSG) and meglumine antimoniate (Glucantime). WHO has suggested 28 days regimen therapy of pentavalent antimonials for the treatment of VL by IV or IM route (Alborzi et al., 2017). However administration of this drug intralesionally (IL) in case of leishmaniasis recidiva cutis also gives positive results by completely purging the papules (Calvopiña et al., 2017). These drugs distort the crucial enzymes of \textit{Leishmania} after transforming from pentavalent to trivalent form (Abamor et al., 2017). Antimonials are allied to far-ranging acute side-effects, the most serious being cardiotoxicity, pancreatitis, nephro-and hepatotoxicity (Atia et al., 2015). They also annihilate the antioxidant defense system of host and thus are involved in the DNA damage of the host itself (Moreira et al., 2017). Another unfortunate trouble with this drug is the emergence of resistant parasite strains. In the antimony resistant species, molecules like nucleoside diphosphate kinase b and elongation factor 2 which are the crucial participants in the metabolism of nucleic acids and proteins respectively get modified (Moreira and Murta, 2016). Other changes like less expression of aquaglyceroporin-1 and over-expression of the various enzymes of thiol metabolism also contribute to mounting of resistance (Perez et al., 2016). Moreover about 58% of cases treated with the SSG progress to the PKDL as reported in Sudan (Salih et al., 2017). Owning to the increased cases of resistance, monotherapy of this drug has been replaced by the combination therapy in which SSG is combined with paromomycin. In Eastern Africa this combination therapy is now been raised to first line treatment option (Kimutai et al., 2017).

Amphotericin B deoxycholate (AmB) is a valuable drug in India (Rodrigo et al., 2017). AmB shows parasitical activity via targeting ergosterol of plasma membrane of the parasite (Mwenechanya et al., 2017). During AmB treatment severe
nephrotoxicity occurs even at the dose of 1 and 0.5 mg/kg as indicated by the high levels of creatinine in the serum. This causes halting of the therapy in between for recovering which further increases the burden on the poor people as it increases the treatment regimen and hence the expenses. Administration of AmB also results in nausea, fever and vomiting (Rabi Das et al., 2017). Moreover changes in the main drug targets like over-expression of cysteine synthase and mutation of sterol 14α-demethylase gene of parasite leads to the drug resistance (Mwenechanya et al., 2017; Singh et al., 2017).

To minimize the toxic effects of AmB the liposomal form was formulated. Liposomal AmB (L-AmB) is approved by FDA as it possesses no side-effects and has extensive leishmanicidal efficacy. It is the most favored chemotherapeutic drug for the treatment of VL in Europe, US and South Asia (Balasegaram et al., 2012; Morizot et al., 2016). The performance and regimen related trial studies in India and Bangladesh showed the highest cure rate of 95.7% in India (Sundar et al., 2010; Sundar et al., 2011; Mondal et al., 2014). L-AmB is better than antimonials and amphotericin B in terms of safety profile and cure rates (Romero et al., 2017) but its high cost, its unreliability at high temperature complicates its use in the field studies (Hendrickx et al., 2017). According to price validated by the WHO and the Drug Regulation Board the estimated charges of treatment at 3mg/kg b.wt. for 7 days come out to be 659.79 and 11,559.15 USD respectively (de Assis et al., 2017). Moreover in some cases as in the travellers with CL or MCL the otherwise wider cure window of L-AmB gets narrowed down (Guery et al., 2017).

Miltefosine (MT), a neoplastic drug, is the sole oral agent to manage leishmaniasis with satisfactory cure rates (Alves et al., 2017) and distinct pharmacokinetics in adult and pediatric patients (Castro et al., 2017). It kills the parasite by binding to the cell membrane in detergent like manner and rupturing it by pore formation (Fernandes et al., 2017) and also by alcalinizat of acidocalcisomes of the parasite (Pinto-Martinez et al., 2017). MT treatment in PKDL shows better output than L-AmB (Moulik et al., 2017). Although MT has high curative rates but mutations of 354TyrPhe and 1078PheTyr in the transporter gene of parasite has resulted in the resistance and relapse cases in India (Srivastava et al., 2017). Such resistant varieties of the parasite proclaim more virulence, less accretion of the drug and alterations in the metabolic pathways which rescues it from the drug
Introduction

(Deep et al., 2017). Besides this despair, the major health concern with MT is its teratogenic nature which has narrowed its application (Alves et al., 2017).

Vaccination is an acknowledged potential and cost-effective strategy to control Leishmania. Many studies endorse the worth of antigens derived from parasite as well as from vector as candidate for anti-Leishmania vaccine (Cecilio et al., 2017). Nucleoside hydrolase, a key antigen of Leishmune® vaccine flaunts encouraging immunizing properties against L. donovani. Its F1 domain harbours maximum specific antigenic behavior in terms of production of protective IFN-γ, TNF-α, IL-17, granzyme B and PBMC’s suggesting its role as vaccine candidate for humans (Carrillo et al., 2017). The saliva of sandfly vector plays pivotal role in the conveyance and establishment of the Leishmania infection. The salivary compounds like Yellow-related proteins (YRPs) augments the VL affliction as exemplified with the high Th2-type cellular immune response in the endemic individuals who are more exposed to the sandfly bites. This immuno-inciting activity of the saliva of the fly has made it a very important anti-leishmanial vaccine candidate (Sima et al., 2016; Kammoun-Rebai et al., 2017). The saliva of L. whitmani has been found to provide robust immunization to the mice with small lesions and low parasite load (Gomes et al., 2017). ChAd63-KH, an engineered simian adenovirus human vaccine expressing two proteins of Leishmania is in the first clinical trial. It generates substantial immune response in relation to IFN-gamma, DC and CD8+ T cells (Osman et al., 2017). Various other vaccines have been designed at IDRI, Sabin Vaccine Institute and the National Institutes of Health but they all are at the stage of clinical trials or only at early research levels with no vaccine available for human leishmaniasis till date (Gillespie et al., 2016).

The eradication project of leishmaniasis has got shattered due to the absence of any prophylaxis measures or proper chemotherapeutic options. In addition to this evolution of resistant species of Leishmania against the standard chemotherapeutic compounds has further complicated the condition (Bouyahya et al., 2018). This bleak situation where control of leishmaniais relies only on the treatment demands discovery of an alternative treatment choices which subdued all the shortcomings of the current treatment modalities. In this regard medicinal plants represent a tremendous reservoir with inherent potential to act as chemo-therapeutants against many diseases (Gachelin et al., 2017). The plants serve as the repository of large
number of diverse bioactive compounds which can substitute the prevalent synthetic drugs (Ullah et al., 2016). The trend of research is projecting more towards finding a natural product as they serve healthy and effective remedies against many clinical manifestations (Al Nasr and Ahmed, 2017). The natural compounds crack the ideology of most dangerous and unbeaten health issues (Kaur et al., 2017). The two foremost antimalarial compounds, quinine (Al Nasr and Ahmed, 2017) and artemisinin (Daddy et al., 2017) have raised the hope and scope of development of anti/protozoal drugs from the plants. Today substantial number of researches have recognized and isolated suitable bioactive compounds which possess antileishmanial potential. There are considerable in vitro and in vivo studies which show ample range of plants which have enormous potential against this catastrophic leishmanial parasite (Ullah et al., 2016). Plants like Bergenia ligulata (Kaur and Kaur, 2018), Cistus crispus, Arbutus unedo (Bouyahya et al., 2018), Inula chritmoides, Spergularia rubra (Oliveira et al., 2017), Sterculia villosa (Das et al., 2017), Cinnamomum cassia, Zingiber zerumbet, Elsholtzia ciliata and Amomum aromaticum (Binh Le et al., 2017), Withania somnifera (Chandrasekaran et al., 2017), Handroanthus serratifolius (Costa et al., 2017), different species of Artemisia, Thymus vulgaris, Allium sativum, Peganum harmala, Achilleamille folium (Soosaraei et al., 2017), Portulaca oleracea, Medicago lupulina (Eskandari et al., 2017), Trillium govanianum (Khan et al., 2017), Schinus molle, Lantana camara and Prosopis laevigata (Delgado-Altamirano et al., 2017), Kalanchoe pinnata, Plumbago scandens, Physalis angulata, Peschiera australis, Piper aduncum, Phyllanthus amarus (de Oliveira et al., 2017), Tinospora cordifolia and Asparagus racemosus (Sachdeva and Kaur, 2017) are some of the noteworthy plants with remarkable leishmanicidal activity. The anti/protozoan propensity of these plants has been attributed to the presence of different groups of biologically active compounds such as alkaloids, terpenoids, phenols, tannins, glycosides, flavonoids and phytosterols (Vishnu et al., 2013; Yadav et al., 2017).

For instance, the juice of Amazon plant, Euterpe oleracea Martius induces apoptotic death in L. (L.) amazonensis and L. infantum through increasing ROS and decreasing levels of IL-17 cytokine (Da Silva et al., 2017). Tricin, an organic component isolated from Casearia arborea eliminates amastigotes of L. infantum by modulating the immune system and by generating reactive oxygen species (Santos et
Ursolic acid, an active compound in *Baccharis uncinella* harbours good antileishmanial activity. It can control the parasite in the spleen and liver via IFN-γ and NO which are known leishmanicidal molecules (Jesus et al., 2017). A sesquiterpene lactone, deoxymikanolide obtained from *Mikania varifolia* and *M. micrantha* possesses encouraging immunostimulatory and leismanicidal favoring activities (Laurella et al., 2017). Similarly acyclic sesquiterpene isolated from *Sapindus saponaria* L. eliminates *Leishmania* via depolarization of the mitochondria, by translocating phosphatidylserine from inner to outside of cell membrane and exposing it to the apoptotic mechanism (Moreira et al., 2017).

In terms of natural products, Propolis is a well-known ingredient of many commercial commodities with medicinal role and is a rich source of active components like flavonoids and triterpenes which are important components in reference to antileishmanial constituents (Cuesta-Rubio et al., 2017). It manifests behavior antagonistic to many species of *Leishmania* by favoring microbicidal cytokine TNF-α and opposes expansion of parasite sustaining TGF-β and IL-10 (Reboucas-Silva et al., 2017). Many other compounds like lapachol (Costa et al., 2017), enhydrin, uvedalin and polymatin B (Ulloa et al., 2017), (S)-cis-verbenol (Yaluff et al., 2017), bergenin (Kaur and Kaur, 2018), dehydrodieugenol (Rodrigues et al., 2016), trans-stilbene, terphenyl derivatives (Bruno et al., 2017) and caeffic acid (Abamor et al., 2017) exhibit potent noxious activity against *Leishmania*. Further plenty of investigations are going on to scrutinize the efficacy of combination therapy involving natural products and standard antileishmanial drugs. For instance, curcumin shows synergistic strength to trigger the secretion of ROS and NO species and escalation of lymphocytes to restraint the growth of *L. donovani* when administered along with miltefosine (Tiwari et al., 2017). Similarly meglumine antimoniate (MA) plus fisetin, a flavonoid displays better ‘cidal’ activity and the combination has far more safety profile than MA alone (Adinehbeigi et al., 2017).

This motivated us to further search medicinal plants and natural products which could serve in the battle against leishmaniasis and could provide novel leishmanicidal agent. In the current study hydroethanolic root extract of *Rhodiola imbricata* Edgew. (HERERI) and its two vital secondary metabolites, Salidroside (SAL) and Rutin (RTN) were explored for their competence against this notorious parasite, *Leishmania donovani*. The antileishmanial efficacy of HERERI
and its bioactive compounds was checked against two different strains of *L. donovani*, i.e. sensitive to sodium stibogluconate (SSG) and resistant to SSG.

*Rhodiola* spp. is indigenous to high elevated areas of Europe, Asia and Northern Hemisphere. It is a succulent perennial plant which belongs to crassulaceae or stone crop family. One of the species of genus *Rhodiola* i.e. *R. rosea* is authorized food supplement as stated by the Ministry of Health. It has stupendous prospective as a therapeutic and pharmaceutical agent in many domains of indisposition of humankind (Jurica and Koupa, 2016; Khanna *et al*., 2017). The roots of *Rhodiola imbricata* Edgew. are the source of nutrients like amino acids, saturated and unsaturated fatty acids and different minerals like iron, calcium and magnesium (Tayade *et al*., 2017). It can be used as a substitute for the antibiotics and can also be exploited as an adjuvant as it has immense potential of stimulating immune system (Kaur *et al*., 2017; Lewicki *et al*., 2017). It flaunts valuable effect on the learning and memory abilities by exciting the neurotransmission of monoamines and by changing the levels of neurotransmitter, acetylcholine. It has pronounced positive reaction even in case of stress and mental disorders (Vasileva *et al*., 2016). Large amount of research work authenticates the function of *Rhodiola* plants in countering the DNA disruption and modulating the expression of variety of chemokines and enzymes related to respiratory burst (Li *et al*., 2017). *Rhodiola* possesses potential immunostimulatory benefits in terms of enhancing Th1 type of cytokines like IFN-γ, IL-2 and IL-12 and in increasing the levels of CD3+ and CD4+ T lymphocytes. It also rescues T cells from the early apoptosis via impeding apoptotic Bcl-2 protein. The immunomodulatory property of this plant is mainly attributed to the glycosides present in it (Liu *et al*., 2015). These properties of *R. imbricata* are mainly accredited to the presence of wide range of secondary active metabolites, most preferential of which are Salidroside and Rutin.

Salidroside (SAL) is a highly valuable phenylpropanoid glycoside found in heterogenous species of genus *Rhodiola* which possesses broad spectrum of medicinal properties (Zduriencikova *et al*., 2018). SAL is a hepatoprotective agent as it guards the hepatocytes from inflammatory response and apoptosis through suppressing MAPK signaling pathway and triggering GSK-3β/Nrf2- riposte (Cai *et al*., 2017; Feng *et al*., 2017). In case of liver damage conferred due to imbalanced immunity, SAL maintains equilibrium in the levels of CD4+ and CD8+ T lymphocytes by
synchronizing CXCL-10 and by shrinking the pursuit of NF-κB (Hu et al., 2014). It possesses cellular protective efficacy as it has the ability to counteract the cell and DNA damaging effects of toxic compound cisplatin by reinstituting the signaling pathways and molecules involved in the survival of the cell at a suitable level (Zduriencikova et al., 2018). SAL evinces anti-cancerous action too against different kinds of tumors like colorectal cancer (Fan et al., 2016), breast cancer (Zhao et al., 2015) and renal cancer (Lv et al., 2016). It promotes the apoptosis of tumor cells by shredding the nuclear material and by abrogating signaling pathways like PI3K/Akt/mTOR. It also increases cell death of tumor cells via ceasing the ratio of Bcl-2/Bax protein and enhancing the autophagic vacuoles and autophagy related gene, Beclin-1 (Fan et al., 2016). SAL is also a potential booster of immune system. It controls many infections via regulating different cytokines and cells of immune system. For instance it competently thwarts the replication and multiplication of dengue virus via increasing the circulating levels of CD8+ T and NK cells (Sharma et al., 2017). SAL plays protective role in asthma provoked due to allergy by conserving the Th1/Th2 balance as it decreases the levels of IL-4 and increases that of IFN-γ (Wang et al., 2014a). The liposomal formation of SAL displays adjuvant activity as it has the ability to provoke the humoral and cell mediated immunity. Moreover it possesses the capacity to induce the maturation of DCs and to enhance their antigen presenting powers (Zhao et al., 2013).

Rutin (RTN) is a natural flavonoid which is also called as vitamin P or rutoside. RTN has well known nutraceutical, therapeutic and pharmacological usefulness (Ganeshpurkar and Saluja, 2017b). It has pronounced hepatoprotective efficacy as it normalizes the deranged liver function markers as well as adjusts the levels of various involved cytokines in standard range (Mansour et al., 2017). It executes through TGF-β/Smad signaling cascade by modulating many proteins of this pathway like Smad-2, Smad-3 and caspace-3 (AlSharari et al., 2016). RTN also shields the liver from the fatal effect of cadmium and ethanol (Abarikwu et al., 2017). It induces humoral immune response, increases DTH response against RBCs of sheep. It also has the ability to rehabilitate the phagocytic capacity of white blood cells (Ganeshpurkar and Saluja, 2017a). Besides these important properties, RTN also exhibits radioprotective (Patil et al., 2017), anticancerous (Aneknan et al., 2014) and neuroprotective (Almutairi et al., 2017) properties.
Rationale Of The Study

VL is an opportunistic disease which mainly afflicts people anguished with impaired immunity. For decades it is the chief cause of mortality in developing countries including India. In respect to the available therapeutic options in the market leishmaniasis is ill-favoured because pharma companies do not gain any incentives or capital in formulating antileishmanial drugs as it is a malady of poor people. Moreover no vaccines or chemoprophylactic options are currently available for the prevention of leishmaniasis. Infact, those drugs which do exist have many pitfalls in terms of price, stability, development of resistance and other severe side-effects. For sustained eradication of leishmaniasis, discovery of a novel immunotherapeutic agent which should be economical, stable and has no cytotoxic effects is of utmost importance. Moreover the exact efficacy of the therapeutic agent can only be confirmed by investigating its safety window along with its potential against various strains of Leishmania including the resistant ones. In this scenario the present study contributes in the field of drug discovery by exploring the phytotherapeutic alternatives for treatment of leishmaniasis. The current study evaluates the efficacy of plant, Rhodiola imbricata and its two active components, Salidroside and Rutin in alleviating the disease through reinforcing the Th1 line of immunity in L. donovani challenged mice. We focused our investigation on finding the immunological properties of the plant and its bioactive components to control this deadly infection as lack of appropriate and significant immunity limits the success of antileishmanial agents and thus leads to progression of disease.

The current study encompasses the following aims and objectives:

1. To explore different phytochemicals of HERERI by qualitative as well as quantitative assays.
2. To identify Salidroside (SAL) and Rutin (RTN) by liquid chromatography-mass spectrometry (LC-MS) or by high performance liquid chromatography (HPLC) in the plant extract.
3. To inspect in vitro antileishmanial activity of HERERI, SAL and RTN against SSG resistant and sensitive strain of L. donovani and then to compute minimum inhibitory concentration (IC₅₀).
4. To determine the *in vitro* toxicity of HERERI, SAL and RTN against the human cell line, THP-1 and to allocate CC$_{50}$. 

5. To examine the *in vivo* toxicity of HERERI, SAL and RTN in mice by limit test of Lorke. 

6. To evaluate the population of promastigotes in sub G$_0$/G$_1$ phase by propidium iodide (PI) staining using flow cytometer. 

7. To enumerate the parasite load by light microscopy and real-time PCR in different groups of animals. 

8. To monitor the delayed type of hypersensitivity responses against leishmanin antigen in different groups of animals. 

9. To evaluate the profile of various cytokines (TNF-α, IL-12, IL-4, IL-10 and IFN-γ) in the sera of different groups of animals. 

10. To perform immunophenotypic analysis and quantification of different immune cells (CD3$^+$, CD4$^+$, CD8$^+$ T and CD19 (plasma B) cells by flow cytometric technique in different groups of animals. 

11. To diagnose the treatment related side-effects by: 
   a. Assessment of important biomarkers of kidney and liver functions 
   b. Histological studies of various organs viz. kidney, liver and spleen. 

12. To check the expression of iNOS and NF-κB genes in infected BALB/c mice via real time PCR after SAL or RTN treatment. 

13. To monitor the levels of leishmanicidal molecules viz. ROS and NO in spleen of SAL and RTN treated infected animals.