Introduction
1. Introduction

Parasitic diseases were always a heavy burden for humanity. Protozoan parasites are responsible for several important diseases that threaten the lives of nearly one quarter of the human population world-wide. Leishmaniasis is a disease complex caused by haemoflagellate obligate intracellular protozoa belonging to the genus *Leishmania*, family *Trypanosomatidae* of the order *Kinetoplastida*.

Designs on pre-Colombian pottery and the existence of thousand year old skulls proves that the disease has been present in the Americans for a long time. It has also been present in Africa and India since the mid eighteenth century (Desjux *et al.*, 2001). It ranks second only to malaria, causing considerable morbidity and mortality in tropical and subtropical regions of the world. The control of leishmaniasis remains a serious problem and with ever increasing cases worldwide, it has become a major focus of concern and a serious Third World problem afflicting the poorer sections of the society (WHO, 2002).

This pathogenic protozoon causes a wide spectrum of clinical manifestations in man and other mammalian hosts ranging from single cutaneous ulcers to mutilating facial lesions and fatal visceral infection. *Leishmania donovani* is the etiologic agent of visceral leishmaniasis (VL), the most severe form, which if left untreated can prove fatal. This is the form prevalent in different parts of India especially in northeast Bihar and eastern Uttar Pradesh.

Chemotherapy constitutes the main line of defense against VL, with organic pentavalent antimonials being the standard first line treatment for more than fifty years. In the present situation, antimonials seem to be losing their efficacy with emerging resistance especially in the state of Bihar where resistance has become endemic. The emergence of unacceptable high level of unresponsiveness to standard antileishmanial drugs posses a major therapeutic challenge.
1.1. Geographical distribution

Leishmaniasis has been attested in 88 countries in five continents—Africa, Asia, Europe, North America and South America (22 in the New World and 66 in the Old World) (Desjeux, 2001), 16 are developed countries, 72 are developing, and 13 of them are among the least developed (WHO, 2005). Approximately 1.5 billion individuals are at risk from this disease and 400,000 cases are reported annually (Herwaldt, 1999, Ashford et al., 1992). There is an estimated prevalence of 12 million (WHO, 1990) with an incidence of 1.0 to 1.5 million cases per annum of the disfiguring cutaneous leishmaniasis (CL) and 0.5 million cases per annum of the potentially fatal visceral leishmaniasis (VL) (Ashford et al., 1992). Over 90% of the global total of VL cases occur in five countries: India, Bangladesh, Nepal, Sudan and Brazil (WHO, 2000, Herwaldt, 1999) and 90% of CL cases occur in 7 countries: Afganistan, Algeria, Brazil, Iran, Peru, Saudi Arabia and Syria. During the past decade there have been epidemics of VL in Sudan (Ashford et al., 1992, Seaman et al., 1996), northeast Brazil (Costa et al., 1990), Bangladesh and the states of Patna and Bengal in India (Bora, 1999). Leishmaniasis is now an emerging zoonosis in the United States (Enserink, 2000, McHugh et al., 2003 and Rosypal et al., 2003) and US soldiers and peace keeping corps currently in the Middle East are experiencing a large outbreak of leishmaniasis with more than 500 parasitologically confirmed cases (CDC, 2004).

1.1.2. Leishmaniasis in the Indian subcontinent

1.1.2.1. Historical background

The rich heritage ancient medical literature of India makes no mention of kala-azar, the popular name of visceral leishmaniasis or any disease entity akin to it. Kala-azar is term coined in India and literally mean ‘black fever’ but could equally mean ‘fatal fever’. The earliest available reference on the subject is contained in the annual report of the Inspector General of civil hospitals for 1872, in which he has quoted the civil surgeon of Burdwen district about the disease. The source from which kala-azar was introduced in Indian subcontinent remains a matter of conjecture. The advent of the disease in Bengal coincides with
periodic incursions and raids by the Portuguese to the area, and it is possible that they introduced the disease either from their own country or from colonies from Africa. Alternative source could have been linkage with China through British maritime traffic. However, the disease in India is not identical with the disease in any of these areas, the nearest resemblance being with the East African form. Once introduced, the condition being appropriate, the disease possible adapted itself and then established a firm foothold.

1.1.2.2. Current situation in India

In the Indian subcontinent, the most common endemic form of the disease is visceral leishmaniasis (VL or kala azar). Kala azar is present in India for more than 100 years. The situation is particularly grave in the state of Bihar, India, known as the "heartland of kala-azar". It has recently posed a serious threat involving 38 out of 42 districts of Bihar state, 8 districts of West Bengal and 2 districts of eastern Uttar Pradesh.

At present the disease is present in almost all districts of Bihar, four districts of Jharkhand, five districts of Uttar Pradesh and 10 districts of West Bengal. 40 out of total 54 districts in Bihar are badly affected with VL. The known highly endemic districts of kala azar are located north of the river Ganges namely Muzaffarpur, Vaishali, Darbhanga, Samastipur, Madhubani, East Champaran, Sitamarhi, Begusarai, Saran, Saharsa and Purnea (Fig.1.1).

In Uttar Pradesh occurrence of sporadic cases of kala azar started in the year 1987 with most of the cases reported so far from this state are imported cases and in West Bengal 9 districts are affected including Malda, Dinajpur and Darjeeling districts (Kar et al., 1999). Kala azar has spread from Bihar to West Bengal and Bangladesh in the east, Uttar Pradesh in the west and Nepal in the north. In 2005 the health ministers of three Member States of WHO's South-East Asia Region, India, Nepal and Bangladesh, had signed a Memorandum of Understanding pledging to collaborate to eliminate VL from their countries.
1.2. Disease and its epidemiology

Leishmaniasis is not a single disease but a variety of syndromes that differ remarkably with one another. The WHO considers Leishmaniasis as one of the most important parasitic diseases (WHO, 1990).

1.2.1. Various forms of Leishmaniasis

Leishmaniasis is a group of diseases with wide epidemiological and clinical diversity. Governed by parasite and host factors and immunoinflammatory responses, the clinical spectrum of Leishmaniasis encompasses subclinical (inapparent), localised (skin lesions), and disseminated infection (cutaneous, mucosal, or visceral) (Kane et al., 2000). According to the form of the disease it is divided into following general clinical patterns.

1.2.1.1. Cutaneous Leishmaniasis (CL)

CL is commonly known as oriental sore. Its causative agents are L. major, L. tropica, L. aethiopica, L. infantum in Old world and L. mexicana, L. venezuelensis, L. amazonensis, L. braziliensis, L. panamensis, L. guyanensis, L. peruviana and L. chagasi are in New world. It produces skin lesions mainly on
the face, arms and legs. It is frequently self-healing in the Old World but, when the lesions are multiple and disabling with disfiguring scars, it creates a lifelong aesthetic stigma. Its most severe form, recidivans leishmaniasis, is very difficult to treat, long lasting, destructive and disfiguring. After recovery or successful treatment, cutaneous leishmaniasis induces immunity to reinfection by the species of *Leishmania* that cause the disease.

1.2.1.2. Diffuse cutaneous leishmaniasis (DCL)

It is difficult to treat DCL due to disseminated lesions that resemble leprosy and do not heal spontaneously. This form is especially related to a defective immune system and it is often characterized by relapses after treatment.

1.2.1.3. Mucocutaneous leishmaniasis (MCL)

It is also called 'espundia' in South America. Causative Agents of MCL in Old world are *L. aethiopica* (rare), *L. major* and in New World are *L. mexicana*, *L. amazonensis*, *L. braziliensis*, *L. guyanensis* and *L. panamensis*. The parasite invades the mucocutaneous region of the body and spread to the oronasal/pharyngeal mucosa. The soft tissues and cartilage of the oronasal/pharyngeal cavity undergo progressive erosion. In contrast to cutaneous leishmaniasis, these lesions do not heal spontaneously. Suffering and mutilation are severe and death occurs as a result of bronchopneumonia or malnutrition. Reconstructive surgery of deformities is an important part of therapy.

1.2.1.4. Visceral leishmaniasis (VL)

It is also known as 'kala-azar'. It is caused by *L. donovani* complex i.e. *L. donovani donovani* (India, Africa), *L. d. infantum* (Middle East and some parts of Asia) and *L. d. chagasi* (South America). Initially, the disease is characterized by high fever, headache, chill, malaise, dizziness, anorexia, vomiting and weight loss. In chronic stage the disease is followed by hepatomegaly, splenomegaly,
lymphoadenopathy, occasional acute abdominal pain, emaciation, anemia, leucopenia, and blackness of skin. (Khalid et al., 1990; ElHag et al., 1994) hence the name given Kala azar or Black fever. It is the most severe form of leishmaniasis and is usually fatal if left untreated. The incubation period can be months or years and, unlike the cutaneous forms of leishmaniasis, it involves the internal organs. After treatment and recovery, the patients may develop chronic cutaneous leishmaniasis that requires long and expensive treatment.

1.2.1.5. Post kala azar dermal leishmaniasis (PKDL)

Post kala azar dermal leishmaniasis is a sequel to the infection with \textit{L. donovani}. Its causative agents in Old world are \textit{L. infantum}, \textit{L. donovani}, and \textit{L. tropica} (rare; also may produce the atypical viscerotropic disease) and in New world \textit{L. chagasi} is responsible for this. It is a type of non-ulcerative cutaneous lesion, developed in about 10\% of kala azar patients generally one or two years after completion of antimonial treatment (Rees and Kager, 1987). PKDL in India resembles lepromatous leprosy with verrucous papilomatous, xanthomathous and gigantic nodular forms (Zijlstra, 1962), while in East Africa it resembles more to sarcoidosis and tuberculosis with popular rash over face or well defined rounded papules (Gasim et al., 1986).

1.3. Vector and life cycle

The leishmaniasis is caused by 20 species, pathogenic for humans, belonging to the genus \textit{Leishmania}, a protozoa transmitted by the bite of a tiny 2 to 3 mm long insect vector, the \textit{phlebotomine} sandfly. It is found throughout the world inter tropical and temperate regions. Of the 500 known \textit{phlebotomine} species, only 30 of them have been positively identified as vectors of the disease. Only the female sandfly transmits the protozoa, infecting itself with the \textit{Leishmania} parasites contained in the blood, it sucks from its human or mammalian host in order to obtain the protein necessary to develop its eggs. In the Old World (Europe, Asia, and Africa) sandfly vectors belong to the genus \textit{Phlebotomus} and in the New World (America), to the genera \textit{Lutzomyia} and
Psychodopygus. Transmission of parasite may be anthroponotic (from one human to another) or zoonotic (from animal to human). In India, the disease is completely anthroponotic where as in certain parts of the world, there are one or more reservoirs (zoonotic host) e.g. dogs in the Mediterranean region and rodents in South Africa.

1.3.1. Life cycle

Leishmaniasis has a digenetic life cycle that alternates between the alimentary tract of the sandfly vector as an extracellular promastigote and in the acidic phagolysosome of macrophages as an intracellular amastigote (Fig.1.2).

![Fig. 1.2. Life cycle of Leishmania](image)

1.3.2. Epidemiology of Leishmaniasis

The epidemiology of Leishmaniasis in a given area is directly dependent on the behaviour of the human and/or animal population in relation to the cycle of
transmission. There are a variety of factors that influence the transmission of the disease. They are as follows (Gebre et al., 1993):

• Proximity of residence to sandfly breeding and resting sites.
• Type of housing.
• Occupation.
• Extent of exposure to sandfly bites.
• Natural resistance, genetic or acquired.
• Virulence of the parasite species.
• Zoonotic or anthroponotic reservoirs. It seems that zoonotic reservoirs are particularly stable when wild uncontrolled populations (e.g. rodents) are involved. Up till now it has been observed that humans are not a reliable agent because of death and treatments except of the chronic condition of PKDL. Nevertheless recent reports about asymptomatic infections in healthy blood donors in France (le Fichoux et al., 1999) are adding a new parameter to the later.
• The vectorial capacity, which is defined as the number of infective bites delivered per human per annum (Dye, 1992).
• Density, seasonality, longevity and flight range of sandfly populations.
• Anthropophilia or zoophilia of sandflies and degree of it.

1.4. Current options for treatment

Despite the considerable progress made in the study of the biochemistry, physiology and molecular biology of Leishmania parasites, the absence of effective vaccines and vector control programs, makes chemotherapy the most widely used tool against leishmaniasis. The current situation for the chemotherapy of leishmaniasis is more promising than it has been for several years with both new drugs and new formulations of old drugs either recently approved or on clinical trial (Croft et al., 1997). The drugs available for treatment of Leishmania infections includes:
1.4.1. Antimonials

In a treatise published in Leipzig in 1604, antimony (Sb), which was introduced by Paracelsus as a general panacea in the 16th century, was acclaimed as one of the Seven Wonders of the World. Sometimes banned and often argued over for another three centuries, the modern era of usage began in 1905 when Plimmer and Thompson showed the activities of sodium and potassium tartrate against trypanosomes in rats and subsequently in the treatment of human trypanosomiasis in Africa. The first published records of use of these trivalent antimonials for treatment were by Macado and Vianna in 1913 for CL, by di Cristina and Cariona in Sicily and Rogers in India in 1915 for VL (Beveridge et al., 1958). The development of the less toxic pentavalent antimonials in the 1920s by Brahmachari, Schmidt, Kikuth and others led to the synthesis of antimony gluconate (Solustibosan) in 1937 and sodium stibogluconate (Pentostam) in 1945 (Goodwin et al., 1958). Another carbohydrate complex, melamine antimoniate (Glucantime, Aventis) soon followed. Out of these two drugs the structure of stibogluconate is still unknown despite its use for 50 years as the first line drug for VL. To be active against Leishmania, SbV has to enter the host cell, cross the phagolysosomal membrane and act against intracellular amastigotes. It is also highly likely that SbV has to be converted to a trivalent form (SbIII) in order to be active, thus leading to the definition of SbV as a prodrug. The details of the activation mechanism and the exact site of this conversion of SbV to SbIII is still unclear. Biochemical studies over the past two decades have indicated a number of potential targets for pentavalent antimonials; glycolysis (Berman et al., 1987), in particular inhibition of ADP phosphorylation (Berman et al., 1985), DNA I topoisomerase (Chakraborty et al., 1988, Lucumi et al., 1998), inhibition of fatty acid beta-oxidation (Berman et al., 1989) and trypanothione (Legare et al., 1997, Mukhopadhyay et al., 1996). Recent studies have shown that both SbIII and SbV mediate DNA fragmentation in Leishmania species, suggesting that antimony kills the parasite by a process reminiscent of apoptosis (Lee et al., 2002; Sereno et al., 2001; Sudhandiran and Shaha, 2003). Long courses of parenteral
administration, variable efficacy against VL and CL, renal and hepatotoxicity and the emergence of significant resistance are all factors limiting the drugs usefulness.

1.4.2. Pentamidine

Pentamidine, an aromatic diamidine, as the isethionate salt (Pentacarinat) and previously as the methylsulphonate salt (Lomidine), have been used as alternative treatments for both VL and CL since 1952. As a second line drug for antimony resistant cases it has proved useful in India and Kenya (Thakur et al., 1991). The efficacy of pentamidine against antimony refractory infections has decreased over the years (Sundar, 2001). Its use has been largely abandoned in India where pentamidine failures are common, but it continues to be used alone or in combination with other drugs in other countries (Basselin et al., 1997, Nacher et al., 2001). Pentamidine was shown to be highly effective against CL in Colombia in a short course low dose regimen (Soto et al., 1994). The antileishmanial mechanism of action of pentamidine remains incompletely understood and there are probably multiple targets, including polyamine biosynthesis (Basselin et al., 1997) and mitochondrial inner membrane potential (Vercesi, 1992 and Angana et al., 2006). Toxicity has always been a limitation on use with reports of hypoglycaemia, diabetes, nephrotoxicity, tachycardia, pain at site of injection (Soto et al., 1994; Jha, 1983). Pentamidine is still used for treatment of haemolymphatic stage of human African trypanosomiasis (Pepin 1994) and, in combination with sulfamethoxazole, for Pneumocytis carinii pneumonitis (PCP) in AIDS patients (Hoover et al, 1993; Montauk, 1992).

1.4.3. Amphotericin B

Amphotericin B (Amp B), a macrolide polyene antibiotic isolated from Streptomyces nodosus was first shown to have antileishmanial activity in the early 1960's and was soon used in the treatment of mucocutaneous leishmaniasis (Furtado, 1960). Amp B is predominantly used as an antifungal drug, specifically for treating systemic mycoses. The selective activity of Amp B
against fungi and Leishmania is due to the higher affinity of the drug for 24-substituted sterols, found in the plasmamembrane of these eukaryotic microorganisms, over cholesterol in the plasma membranes of mammalian cells. Although Amp B has long been considered as an alternative treatment for MCL and VL (WHO, 1996), its use has been restricted by infusion related and delayed toxic side effects, in particular cardiotoxicity and nephrotoxicity (Khoo, et al., 1994). However, antileishmanial chemotherapy has benefited from the development of lipid-associated formulations of Amp B, which have reduced toxicity and an extended plasma half-life in comparison to the parent drug. The formulations in either lipid (AmBisome®, involving unilamellar liposomes, and Abelcet®, incorporating a lipid complex) or cholesterol (Amphocil®, as a colloidal dispersion) have all been in clinical trials for VL and/or MCL. AmBisome® is the best tested of these formulations, has proved to be effective (Berman et al., 1987).

Although resistance to Amp B is rarely reported in eukaryotic microorganisms, it has been possible to generate resistant L. donovani promastigotes in culture (Mbongo et al., 1998; Espuelas et al., 2000). Promastigotes, 20 fold more resistant to Amp B than wildtype, had reduced membrane fluidity, a change from unsaturated to saturated fatty acids (in particular stearic acid) with the major sterol being cholesta-5,7,24-triene-3b-ol, the ergosterol precursor (Mbongo et al., 1998).

1.4.4. Paromomycin (aminosidine)

Paromomycin (PM), an aminoglycoside antibiotic, was originally identified as an antileishmanial in the 1960s and has been used in clinical trials for both VL and CL. Although development of the parenteral formulation of PM, a drug with poor oral bioavailability, for VL has been slow, several Phase 2 trials in India and Kenya have been promising, with 90% of patients cured of VL. There are also encouraging findings on the use of PM as a topical treatment for CL. The report by EIOn and colleagues in 1984 that a topical formulation containing 15% PM and 12% methyl benzethonium chloride (a skin penetrating
agent) was effective against experimental CL led to clinical trials. Hydrophillic PM formulation was found more effective than hydrophobic formulation (Gonçaalves et al., 2005). Combinations of paromomycin with antimonials have been effective against VL (Thakur et al., 2000) with the potential shortening of treatment. It acts by interfering with the mitochondrial activity and inhibiting cell respiration and lowering the electric potential difference across the mitochondrial membranes (Maarouf et al., 1997). Some studies indicated that binding to ribosomal units might be involved as paromomycin promoted subunit dissociation of both cytoplasmic and mitochondrial ribosomes (Maarouf et al., 1997).

1.4.5. Allopurinol and purine analogues

The antileishmanial activity of the purine analogue allopurinol was identified over 30 years ago and, because it had oral bioavailability and was widely used for other clinical indications, it entered clinical trials for VL and CL. All parasitic protozoa studied to date are unable to synthesize purines de novo and, therefore, rely upon uptake and salvage of preformed purines for survival. Allopurinol is used as a substrate by various enzymes of the purine salvage pathway of trypanosomatids, and is selectively incorporated into nucleotide intermediates and nucleic acids in the parasite. In recent years, allopurinol has been considered as part of a maintenance therapy for canine leishmaniasis, against which it has suppressive activity (Koutinas et al., 2001). Clinical trials of allopurinol in combination with other drugs have shown more promising results.

1.4.6. Azoles

Azoles such as ketoconazole, itraconazole, fluconazole, as antileishmanial agents has also been studied (Bahamdan et al., 1997, Salmanpour et al., 2001). The mechanism of action of ketoconazole against Leishmania promastigotes is the same as for Candida albicans, i.e., interference with membrane permeability (cytochrome P450) secondary to loss of desmethyl sterols and accumulation of 14α-methyl sterols. These sterols have a detrimental effect on the membrane permeability and hence on the viability of the organism. Thus, the working
hypothesis is that the accumulation of 14-methylsterols consequently leads to an alteration in the membrane fluidity and permeability. The azole compounds have inevitably weaker action than amphotericin because they act in the synthesis of ergosterol by inhibiting the demethylation of lanosterol (Urbina, 1997). These have not been consistently effective when used alone for the treatment of VL, and are employed in combination with allopurinol or pentavalent antimonials. Fluconazole has, however, been successfully used for CL infections caused by *L. major* (Alrajhi *et al.*, 2002).

### 1.4.7. Miltefosine

Perhaps the most significant recent advancement has been the effective oral treatment of VL by using miltefosine, an alkylphosphocholine originally developed as an anticancer drug (Croft *et al.*, 2003). The antileishmanial activity of miltefosine was initially discovered in the mid 1980s. After a Phase 3 trial, in which 282 out of 299 (94%) VL patients were cured with an oral dose of 2.5 mg kg$^{-1}$ of miltefosine daily for 28 days (Sundar *et al.*, 2002), miltefosine was registered in India for oral treatment of VL and is now in phase IV trial (Croft *et al.*, 2006). It remains to be seen whether miltefosine has similar efficacy against VL in other endemic areas such as Sudan, and against *L. infantum* (also known as *Leishmania chagasi*) VL in South America and the Mediterranean area. Miltefosine has also proved to be active against CL in a clinical trial in Colombia (Soto *et al.*, 2001) and further trials against this disease type are planned. The major limitation of miltefosine is teratogenicity and this excludes its use in women of child-bearing age. The long half-life of miltefosine (2–3 weeks) and its narrow therapeutic index might favour the emergence of resistant mutants. Due to the recent introduction of miltefosine in the field, not too many clinically resistant parasites have been reported. But preliminary data from a phase IV trial in India involving domiciliary treatment with miltefosine and weekly supervision suggests doubling of the relapse rate (Sundar *et al.*, 2005); this provides warning that drug resistance could develop quickly and plans are required to prevent it (Croft *et al.*, 2006). However, resistance can be easily induced in vitro and some of the
resulting mutants have been characterized (Perez-Victoria et al., 2003; Seifert et al., 2003). *Leishmania donovani* promastigotes having mutation in putative miltefosine transporter were shown to be responsible for the reduced uptake of miltefosine. The potential relevance of these observations needs to be extended to miltefosine resistant amastigotes before clinical implications to be made. Combination therapy should be considered, to delay the emergence of miltefosine resistance, particularly in anthropopotic foci where resistance could quickly spread (Bryceson, 2001). The price of the drug is currently under discussion, but to be useful in endemic countries it should be in the range of, and preferably lower, than that of current first-line treatment options.

1.4.8. Immunomodulation

Cure of leishmaniasis, probably even during chemotherapy, appears to be dependent upon the development of an effective immune response that activates macrophages to produce toxic nitrogen and oxygen metabolites to kill the intracellular amastigotes (Berhe et al., 1999; Murray et al., 1989 and Alvar et al., 1997). Studies showed that biological immunomodulators such as interferon (IFN)-γ can provide a missing signal and enhance the activity of antimonials in the treatment of VL and CL. Recently, a new generation of immunopotentiating drugs have shown potential for leishmaniasis treatment. The imidazoquinoline imiquimod, an ingredient of the topical cream for genital warts known as Aladara™, induces nitric oxide (NO) production in macrophages was shown to have antileishmanial activity via macrophage activation in experimental models (Buates and Matlashewski, 1999) and in clinical studies on CL in combination with antimonials (Arevalo et al., 2001).

1.4.9. Natural products

Natural products are often overlooked in antiprotozoal chemotherapy. However, many antimicrobial antibiotics are also important antiprotozoal drugs or have provided important leads in this area. In a recent review (Rocha et al., 2005) 101 plants, their families, and geographical distribution, the parts utilized,
the type of extract and the organisms tested are discussed. It also includes 288 compounds isolated from higher plants and microorganisms, classified into appropriate chemical groups. Licochalcone A from the Chinese liquorice plant *Glycyrrhiza* has shown reasonable oral efficacy in experimental models of VL and CL; synthetic oxygenated derivatives are also active (Zhai *et al*., 1999). One derivative, 35 m4ac, resulted in 97% suppression of *L. donovani* liver amastigotes in a hamster model when given at 20 mg kg$^{-1}$ for six days intraperitoneally. The compounds appear to interfere with mitochondrial function. The 2-substituted quinoline alkaloids, from the Bolivian plant *Galipea longiflora*, have also shown oral activity in experimental VL and CL mouse models (Fournet *et al*., 1996). Saponins purified from the Vietnamese plant *Maesa balansae* and designated PX-6518 showed excellent activity after parenteral administration against VL and CL in rodent models (Olliaro *et al*., 2002).

1.4.10. Combination therapy in VL

Growing resistance of the parasite to antileishmanial drugs suggest that the currently used monotherapy needs to be reviewed. Multidrug combination treatment of VL, as practiced in tuberculosis and leprosy, should be given serious thoughts to prevent/delay the appearance of drug resistance. Although at present not many effective antileishmanial drugs are available, once oral miltefosine (which has been approved for the treatment of VL in India), sitamaquine and parenteral aminosidine become available, these drugs along with Amp B and SbV should be used in combination not only to combat drug resistance but also to shorten the duration of treatment. Thus, the northern districts of Bihar have the distinction of being unique in terms of large-scale SbV failure; resistance is likely to go up and spread into the areas where SbV is still effective. Rampant misuse of SbV (inadequate doses and insufficient duration) has led to the development of refractory strains, which tolerate several times more drug than those still responsive to it. More work is needed to identify the changes occurring in these strains and molecular tools for identification of these strains need to be developed. In areas with SbV resistance, it needs to be replaced with safer and
more effective drugs. Unfortunately at the moment despite its limitations, amphotericin B is the only option for these patients. Oral miltefosine, which has been approved for the treatment of VL in India and should then be, used as first line therapy (Sundar et al., 2005). When more clinically effective antileishmanial drugs are developed, combination chemotherapy may hold the key to curing the emergence of drug resistance.

1.5. Scope of the study

Control of leishmaniasis is very difficult and challenging. Despite impressive advances in science, technology and medicine, we have until now not been successful in allocating sufficient resources to fight this dreadful disease that particularly affects the poor. Although drug management in leishmaniasis has evolved rapidly and with success, but obstacles continue to limit the impact of these advances in regions of endemicity (Murray, 2001). Lack of affordable new drugs, still a basic unsolved problem, has been joined by additional therapeutic obstacles including large scale resistance to pentavalent antimony (SbV) in India and coinfection with human immunodeficiency virus in all endemic regions. Available treatment options have actually expanded and includes successful application of less expensive generic SbV; rediscovery of the high level efficacy of amphotericin B; implementation of shortcourse parenteral regimens (lipid formulations of amphotericin B); potential to replace SbV and amphotericin B with price capped paromyomycin; and identification of the first effective oral agent, miltefosine. How to sustain and move this progress ahead remain difficult next steps in the treatment of leishmaniasis.

There is an urgent need for more selective and efficacious drugs, for that matter, identification of potential drug targets for antileishmanial therapy that are unique to the parasite.