1. INTRODUCTION

Malaria has been a major cause of human sufferings since dawn of mankind. Despite important advances in our understanding of the disease, it still remains a major cause affecting the health and wealth of nations and individuals alike. The magnitude of malaria in terms of morbidity and mortality can be realized from the fact that approximately 40% of the world’s population, mostly those living in the world’s poorest countries, is still at the risk of malaria (Guadalupe et al., 2007; Sahu et al., 2008).

Malaria is caused by an apicomplexan protozoan parasite of genus *Plasmodium*. Twenty species of *Plasmodium* infect primate hosts, 19 rodents and bats and about 70 species have been reported in birds and reptiles. Four species of *Plasmodium* known to infect human beings are – *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae* (Greenwood et al., 2008). Polymerase Chain Reaction (PCR) study has now confirmed the presence of two more species *P. knowlesi* and *P. cyanomolgi* capable of human infection. Earlier infection with *P. knowlesi* was regarded as a rare disease, occurring only sporadically in humans (Coatney, 1963). The recent findings of a large number of infected patients in Myanmar (Zhu et al., 2006), Malaysia (Vythilingam et al., 2008), Philippines (Luchavez et al., 2008), Singapore (Ng et al., 2008) and especially in Sarawak, Thailand (Jongwutiwes et al., 2008) have changed the perception of *P. knowlesi* thus elaborating the severity of malaria. Out of these species, *P. falciparum* and *P. vivax* account for 300-500 million clinical cases and 1.5-2.7 million deaths each year (Sauerwein,
Almost half of the world’s population, spanning 107 countries and territories, live in malaria-prone areas. The geographic distribution of the disease is widening with infection being noticed in previously uninfected regions (Kelly-Hope and McKenzie, 2009). Endemic transmission takes place in most tropical latitudes and reaches into temperate zones seasonally. Although each type of infection causes debilitating febrile illness, *P. falciparum* carries a substantial risk of death.

Approximately 1-3 million deaths occur each year in region of holoendemic infection in Sub-Saharan Africa (Dellicour *et al*., 2007). Most of them are infants and young children below the age of five years (Lusingu and Von Sceidlein, 2008). In addition to children, high-risk groups include pregnant women and non-immune travelers such as armed forces, labourers and refugees. Pregnant women are particularly susceptible to severe malaria and are more likely to deliver underweight babies (Agomo *et al*., 2009).

In India, the North-Eastern areas are highly endemic for malaria claiming more than 1000 lives annually but the epidemic for the cerebral malaria is also observed in unusual areas of Rajasthan and Haryana (Dash *et al*., 2008). Fifty four million individuals of various ethnic origins, accounting for 8% of the total population of India, contributed 30% of total malaria cases, among which 60% cases of *falciparum* contributes 50% malaria deaths in India (Singh *et al*., 2009). Malaria is directly linked to the socio-economic development. In recent years, malaria has become an immense threat to the Indian economy causing crisis situation in Kolkata, Surat, Mumbai, Ahmedabad, Chennai and Delhi (Yadav *et al*., 2003). In terms of disability-adjusted life (DALYS), malaria contributes to an estimated loss of 132 million DALYS annually (Snow *et al*., 2005). Economists believe that malaria is responsible for a growth penalty of upto 1.3% per year in some African countries (Sachs and Malaney, 2002; Teklehaimanot and Mejia, 2008). *Anopheles* is vector for
human malaria (Ross, 1898). There are about 380 species of *Anopheles* mosquito, but only 60 or so are able to transmit the parasite. In India, the epidemiology of malaria is complex because of geo-ecological diversity, multiethnicity and wide distribution of anopheline vectors, transmitting three plasmodial species — *P. falciparum*, *P. vivax* and *P. malariae*. *Anopheles culicifacies* is widely distributed and is the principal vector of rural malaria. *An. stephensi* is the primary urban vector and *An. fluviatilis* is the vector in the hills and foothills, whereas, *An. minimus, An. nivipes, An. philippinensis* and *An. durus* are vectors in the North East. *An. sundaicus* is restricted to Andaman and Car Nicobar islands. *An. annularis* and *An. varuna* are secondary vectors with wide distribution (Kumar *et al.*, 2007).

**LIFE CYCLE OF PLASMODIUM**

The life cycle of *Plasmodium* is very long and highly complex which begins and ends in the female *Anopheles* mosquito (Fig. 1.1). The sporozoites are injected with a mosquito bite. Within an hour, sporozoites enter hepatocytes and begin to divide into exoerythrocytic merozoites (tissue schizogony). For *P. vivax* and *P. ovale*, dormant forms called hypnozoites typically remain quiescent in the liver until a later time, however, *P. falciparum* does not produce hypnozoites. After leaving the liver, merozoites invade erythrocytes and develop into early trophozoites, which are ring shaped, vacuolated and uninucleated. Eventually, the infected erythrocytes are lysed by the merozoites after blood schizogony. The duration of erythrocytic schizogony in *P. falciparum* is about 48 hours. In non-immune humans, the infection is amplified about 20-fold each cycle. After several cycles, some of the merozoites develop into gametocytes, which are ingested by the blood feeding mosquitoes. In the mosquito gut, the gametocytes emerge as gametes and fertilize to produce motile ookinetes, which burrow into the gut wall of
mosquito to form oocysts. Finally, sporozoites are released into the body cavity which ultimately find their way to the salivary glands and are injected to a new host during the next blood meal.

Fig. 1.1: Life cycle of the human malaria parasite showing the sexual (in the mosquito) and asexual (in the human host) phases of life cycle [Adapted from Centre for Disease Control and Prevention].

RODENT MALARIA PARASITES

Rodent malaria parasite is an important tool to study malaria parasite. The four identified species of Plasmodium causing rodent malaria are *P. berghei*, *P. yoelii*, *P. chabaudi* and *P. vinckei*. All species can be grown in laboratory mice and young rats and these can be transmitted through mosquito particularly *A. stephensi*. These parasites have proved to be analogous to the malaria parasites of man and other primates in most essential aspect of structure,
physiology and life cycle. Genome organization, housekeeping genes and biochemical processes between rodents and human parasites are conserved (Carter and Diggs, 1977). Among these rodent parasites, \textit{P. chabaudi} is an ideal model for studying drug resistance in \textit{P. falciparum} (Carlton et al., 2001). \textit{P. yoelii} is preferred for liver stage immunological studies (Ono et al., 2007). \textit{P. berghei} is employed as a useful tool for studying the activity of potential antimalarial drugs (Cruz et al., 2000). A number of genetically modified \textit{P. berghei} lines expressing green fluorescent protein (GFP), are one of the important tools to study and visualize parasites in living host (Amino et al., 2005). \textit{P. berghei} was first isolated from the blood of thicket rat and was described by Vinckei and Lips in 1948. \textit{P. berghei} has \textit{Grammomys surdaster} and \textit{Leggada bella} as natural hosts and \textit{Anopheles dureni} as vector. \textit{P. berghei} has a preference for reticulocytes but also invades mature red blood cells. The blood stage development (6-8 hours) of \textit{P. berghei} in laboratory rodents is asynchronous (Fig. 1.2).

\textbf{Fig. 1.2: Life cycle of Plasmodium berghei}

Source: (Homepage.ed.ac.uk/sreece/fertins.html)

The infection rate of sporozoites in liver cells is different in different species of laboratory rodents. Sporozoites develop via
trophozoite stage to schizonts in 47-52 hours. Gametocytes sequester preferentially in blood capillaries from which mosquitoes take their blood meal (Landau and Chabaud, 1994).

SYMPTOMS OF MALARIA

The clinical symptoms of malaria are primarily due to schizont rupture and destruction of erythrocytes. Malaria can have a gradual or a fulminant course with nonspecific symptoms. According to symptoms and consequences, malaria can be classified into two types namely uncomplicated and severe malaria. The classical symptoms of uncomplicated malaria include cyclic occurrence of sudden coldness followed by sweat, rigour and fever (Genton and Acremont, 2005). These symptoms occur after every two days for *P. vivax* and *P. ovale* infections and every three days for *P. malariae* (Collins and Jeffery, 2007). *P. falciparum* have either continuous or recurrent fever every 36-48 hours. The majority of patients experience cyclic fever, shivering, chills, headache and diaphoresis (Genton and Acremont, 2005). Severe malaria is caused by *P. falciparum*. The symptoms include splenomegaly, severe headache, cerebral Ischemia, cerebral malaria, hepatomegaly, hypoglycemia, haemoglobinuria and renal failure. The presentation of malaria resembles with common viral infections, which often leads to delay in diagnosis (Trampuz *et al*., 2003). If left untreated, severe malaria causes 100% mortality.

DIAGNOSIS OF MALARIA

Correct diagnosis and prompt treatment are of paramount importance in determining the outcome of malaria treatment (Hanscheid and Grobusch, 2002).

Light microscopy of thick and thin blood smears remains the standard method for diagnosing malaria (Warhurst and Williams,
Three fluorescent techniques have promise for the diagnosis of malaria: quantitative buffy-coat (QBC) method, which is available as a commercial kit (QBC: Becton Dickinson, Franklin Lakes, NJ) (Baird et al., 1992; Benito et al., 1994; Gay et al., 1994; Clendennen et al., 1995), Kawamoto Acridine-Orange process (Kawamoto, 1991a,b; Kong and Chung, 1995; Bosch et al., 1996; Gay et al., 1996; Lowe et al., 1996) and benzothiocarboxypurine (BCP) procedure (Makler et al., 1991; Cooke et al., 1992). These techniques are rapid, relatively easy to perform and demonstrate high specificity and sensitivities.

Another approach to the laboratory diagnosis of malaria is based on the detection of nucleic acid sequences specific to the Plasmodium parasites. Two parasite antigens currently used in the new and rapid diagnostic tests include histidine-rich protein-2 (HRP-2), which is only produced by P. falciparum (Howard et al., 1986; Rock et al., 1987) and the parasite lactate-dehydrogenase (pLDH) antigen, which is produced by all four Plasmodium species infecting man. Both these antigens are secreted into the blood by all asexual stages of the parasite. The pLDH antigen is also produced by gametocytes (Oduola et al., 1987). The latest antigen-capture tests are rapid and simple to perform and have detection limits comparable with those of high-quality microscopy (i.e. 100-200 parasites/ml) (Dietz et al., 1995; Palmer et al., 1998).

HRP-2 based tests are 90% sensitive, when there are 60-100 parasites/ml. Commercially there are two such tests available – the ParaSight F test (Becton Dickinson, Franklin Lakes, NJ) and the immunochromatographic test (ICT) (ICT Diagnostics, Sydney). However, neither of these tests is presently available because Food and Drug Administration has not approved them yet. Both these tests detect P. falciparum malaria, which are based on monoclonal antibodies to HRP-2.
The newest rapid serological tests for the diagnosis of malaria are based on the detection of parasite lactate dehydrogenase (pLDH). Since pLDH is only produced by viable parasites, they are also useful in monitoring antimalarial therapy. The rationale behind developing a test that detects pLDH was based on the biochemistry of the enzyme. Parasite lactate dehydrogenase (pLDH) is a unique glycolytic enzyme present in all malarial parasites of human. Furthermore, pLDH from *P. falciparum* can be differentiated from host lactate dehydrogenase (hLDH) with 3-acetylpyridine adenine dinucleotide (APAD), an analogue of nicotinamide adenine dinucleotide (NAD) (Makler and Hinrichs, 1993). The pLDH-based assays are available in two formats – a semi-quantitative, dry, dipstick (OptiMALO; Flow Inc., Portland, OR) and a 96-well, quantitative, immuno-enzymatic, capture assay (Piper *et al*., 1996). The latter assay captures pLDH with an antibody and then quantifies the amount of enzyme captured with an enzyme reaction utilizing APAD. This assay uses two monoclonal antibodies, one specific for *P. falciparum* and the other recognizing all four *Plasmodium* spp. infecting humans.

**MALARIA CONTROL**

Malaria control efforts include attempts to develop an effective vaccine, to eradicate mosquito vectors, and development of newer antimalarial drugs.

**Vector Control**

The vector control is one of the essential components of any malaria control programme, the success of which relies on the knowledge of the vector species, their bionomics and vector control options suitable for vector species. To control the vector, various physical, chemical and bio-control measures have been employed. Insecticides such as organophosphates, chlorinated hydrocarbons
like dieldrin, benzene hexachloride and synthetic pyrethroids such as
deltamethrin, cyfluthrin etc. were utilized to control the mosquitoes.
Trials of insecticide-treated bed nets in last two decades reduced
deaths in young children significantly in Africa. Bed nets’ use in
Kenyan children under 5 years rose dramatically from 7% in 2004 to
67% in 2006 but the emerging pyrethroid resistance in vectors is a
serious threat to the success of pyrethroid-treated bed nets
(Munhenga et al., 2008). Pyrethrum, which is a natural mixture of six
insecticidal esters, have shown the potential to be an alternative
candidate for the impregnation of mosquito nets and textiles in areas
where resistance to pyrethroids has become problematic (Duchons et
al., 2009).

Biological control of mosquito larvae has also been employed
to combat the vector. Leaf extracts of Melia azedarach, Ocimum
sanctum and Leucas aspera contain compounds that have been
found highly toxic to mosquito larvae and exhibit highly deleterious
effects on adult mosquitoes (Nathan et al., 2006). Rearing of
larvivorous fish species like Poecilia reticulata, Gambusia affinis,
Northobrachus spp. and Tilapia spp. were carried out to target the
aquatic stages of mosquitoes (Chandra et al., 2008). The National
Vector Borne Diseases Control Programme is presently implementing
this strategy as part of integrated disease control in many states
(Dash et al., 2007). Two biocides from the bacterium, Bacillus
thurienginesis var. israelensis (Bti) and Bacillus sphaericus (Bs)
have been used in the field for many years, however, Bs has developed
resistance soon after its application (Federici et al., 2007). Virulent
fungi, infecting A. gambiae, has been found to reduce malaria
transmission intensity in Tanzania (Schulte et al., 2005). Yet another
strategy being considered is the genetic manipulation of vectors by
introducing foreign genes into the vector (Terenius et al., 2008).

Malaria Vaccines

Mutation in vector and parasite’s resistance to the drugs
emphasize the need for vaccine. Efforts to develop a vaccine have
been thwarted by the complexity of the parasite's life cycle and the
ability of the parasite to suppress and evade the immune response.
There are six targets for a malaria vaccine development, which
includes – 1) sporozoites; 2) liver stage; 3) merozoites; 4) infected
RBCs; 5) parasite toxins; and 6) sexual stages. The early work with
radiation-attenuated sporozoites, which includes circumsporozoite
protein (CSP), demonstrated high level of protective response in
experimental rodents and primate models (Nussenzweig and
Nussenzweig, 1989). Trials in humans showed that a malaria vaccine
targeting the sporozoite invasion in the hepatocytes should elicit very
high antibody titer, which should be continuously maintained through
induced booster dependent effect, as sporozoite remains in the blood
stream for a very short period of time (Herrington et al., 1991). This is
a major drawback in the hepatocyte invasion blocking rationale.

Liver stage antigens (LSA) represent the second target for a
malaria vaccine. Efforts are being made to improve immunogenicity
and protective efficacy of RTS by using new adjuvant formulation and
comparing oil in water MPL/QS21 (AS02) with MPL/QS21 (AS01B).
Other identified liver stage antigens include SALSA and STRAP
along with liver stage antigen-1 (LSA-1), LSA-2 and LSA-3. Clinical
trials for vaccine containing LSA-1 and LSA-3 are under
investigation. ICC-1132 is recent pre-erythrocytic candidate in
combination of CSP with virus like particle (HBcAg) in trial phase II
has been found to be immunogenic, which elicit CD4+ T cell response
(Nardin et al., 2004).

Beside the sporozoites, the merozoite is only stage in the
human host in which the malaria parasite is extracellular. Several
merozoite surface proteins (MSPs) have been identified, out of which
MSP-1 has been the most investigated blood stage vaccine
candidate antigen. The rhoptry protein apical membrane antigen-1
(AMA-1) and rhoptry associated protein-1 and 2 (RAP-1 and RAP-2)
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are among best studied. Inside the RBC, *Plasmodium* escapes the host's immune response. But CD4+ T cells act through the release of cytokines that may exert parasiticidal or parasitostatic effect, activate macrophages and provide help mainly for antibody production by B cells. Several molecules have been identified that could elicit antibodies, which includes ring erythrocyte surface antigen (RESA), the serine-rich protein (SERP) along with erythrocyte membrane protein-1 (EMP-1) (Leech *et al.*, 1984), EMP-2 (Magowan *et al.*, 1995) and EMP-3 (Pasloske *et al.*, 1993). *Pf*EMP-3 is erythrocyte membrane protein expressed not only on the RBC surface but also by the liver stage and by the sporozoite (Gruner *et al.*, 2001), suggesting that it may have an important role during all the developmental stages of the parasite. These characteristics make it a potential candidate for a malaria vaccine.

The last possible target for a malaria vaccine is the sexually differentiated parasite stages, in an approach called transmission-blocking vaccines (Carter, 2001). Antibodies developed against some proteins of the sexual stages may block the development of the parasite in mosquitoes. The transmission-blocking vaccine is intended to be administered in association with an antiparasite vaccine. Several candidate antigens have been identified and evaluated. The leading candidates are Ps25, Ps28, Ps48/45 and Ps230 (Carter *et al.*, 2000). Pfs25 and Pvs28 antigens are in Phase I and preclinical trails respectively. Pfs25 has been included in multiantigen and multistage vaccines (Ockenhouse *et al.*, 1998). A recent study shows that infection with *P. berghei* boosts antibody responses primed by a DNA vaccine plasmid encoding gametocyte antigen Ps48/45 (Diana *et al.*, 2006). Malaria parasite shows multiple antigenic variations. Thus a single stage vaccine cannot be effective. Hence multiantigen and multistage approaches are being developed. One of the most studied candidates is SPf66 – a synthetic polymer that combines portions of three blood-stage antigens linked to each other by the NANP peptide derived from CSP. Despite the first trials
in Colombia resulting in high protection (Patarroyo et al., 1987, 1988), other extensive field trials thereafter in Latin America, Africa and Asia failed in confirming those results. A multiple antigen peptide (MAP) construction containing several T- and B-cell epitopes of CSP has been tested in mice and three monkey species (Moreno et al., 1999). The MAP construction in association with alum adjuvant concomitant use of another immunostimulant, QS-21, resulted in improved immunogenicity (Nardin et al., 2004). A different approach is the use of viral vectors containing genes coding for Plasmodial antigens. NYVAC-Pf7 is a pox-vectored multiantigen, multistage vaccine candidate. It utilizes the attenuated strain of vaccinia virus expressing genes coding for proteins expressed during the sporozoite (CSP and TRAP), liver (LSA-1), blood (MSP-1, SERP, AMA-1) and sexual (Pfs25) stages of the parasite's life cycle. In a Phase I/II trial, only one of the 35 vaccinated volunteers was protected and overall there was a delay in the patency (Ockenhouse et al., 1998). Human vaccine trials have been conducted with combinations of recombinant MSP-1, MSP-2 and RESA in the Montanide ISA720 adjuvant, with the most recent being a Phase II b trial in children in a highly endemic area of Papua New Guinea (Genton et al., 2005). A hybrid molecule containing the blood-stage antigens SERP, MSP-1 and HRP-2 has been tested in Aotus and the results were controversial in two different studies, with protection being showed in the first (Knapp et al., 1992) but not in the second (Kocken et al., 1998). Recently different multiple stage vaccine (peptide recombinant in combination with alum, heterologous prime boosts, DNA plasmids, recombinant virus) has undergone phase trial II. Peptide recombinant (CSP, NANP-19) and recombinant protein (CSP/MSP-2) in combination with alum shows no protection (Struchler et al., 1995). A combination of CSP, MVA/RTS, AS02A based upon heterologous prime boost strategy shows similar results as that of RTS. Safety, immunogenicity and efficacy of DNA and modified vaccinia virus Ankara (MVA) prime-boost regimes were
assessed by using either thrombospondin-related adhesion protein (TRAP) with a multiple-epitope string ME (ME-TRAP) or the circumsporozoite protein (CS) of *Plasmodium falciparum*. The vaccines were well tolerated and immunogenic, with ME-TRAP DNA/MVA and ME-TRAP/fowl pox virus. Prime-boost vaccination with DNA and MVA encoding ME-TRAP resulted in twenty percent protection against *P. falciparum* sporozoite challenge (Bejon *et al.*, 2005; Keating *et al.*, 2005). A recombinant subunit protein malaria vaccine containing RTS, S/AS02 was designed to block infection against *Plasmodium*. It demonstrated 35% efficacy against uncomplicated malaria and 49% efficacy against severe malaria for at least 18 months in young children and 66% efficacy against *P. falciparum* in infants (Alonso *et al.*, 2005; Aponte *et al.*, 2007). Results of Phase 1 randomized controlled trial of AMA-1 malaria vaccine (FMP2.1/AS02A) have reported good safety profile, well tolerability and high immunogenicity of vaccine against *P. falciparum* in adults. Phase 1 and Phase 2 trials in children are undergoing with this vaccine in West Africa (Thera *et al.*, 2008). Despite considerable efforts, no successful vaccine against malaria has been developed so far.

The flexibility of the DNA vaccine technology permits the combination of several antigens from different developmental stages of the parasite's complicated life cycle. However, at present, there is no malaria vaccine that can be readily used in routine practice for prevention of the disease. All the vaccines till date are under different phase trials either in the clinics or in the fields. The search for a vaccine has been plagued by a number of shortcomings. Many of them are related to antigenic variations, antigenic diversity and immune evasion mechanisms exhibited in various stages of complex life cycle of malaria parasite. While efforts are being made to overcome the hurdles for vaccine development, people are dying and only available effective means of reducing the number of deaths is the provision of affordable and effective medicines.
Chemotherapy

In absence of potent vaccines, chemotherapy remains the main prospective for the treatment of malaria to rely on. Drugs used for the treatment of malaria do not assist natural healing process of the body. Instead they act chemically on the parasite and kill it. In most cases antimalarial drugs target the asexual erythrocytic stage of parasite. The parasite degrades haemoglobin in its acidic food vacuole, producing free haemoglobin able to react with molecular oxygen and generating reactive oxygen species as toxic by products. A major pathway of detoxification of haem moieties is polymerization of haem, thus killing the parasite with own toxic metabolic waste. Antimalarial drugs fall into several groups.

The first and most commonly used antimalarial drugs are quinoline based antimalarials, which include quinine and its derivatives like chloroquine, amodiaquine, mefloquine and halofantrine. Quinine has been used for more than three centuries and until 1930s, it was only effective drug for the treatment of malaria. Quinine has been derived from the bark of *Cinchona* plant. Of the 36 alkaloids found in the bark of *Cinchona* plant only four possess antimalarial properties, with quinine being the most effective. Quinine binds effectively with blood proteins and forms complexes, that are toxic to malaria parasite. It has many undesirable side effects.

Chloroquine is the most important and widely used drug of quinoline series. It was introduced in 1944 and soon became mainstay of therapy and prevention, since this drug was cheap, non toxic and effective against all strains of parasite (Trape, 2001). It is capable of blocking polymerization of haem to haemozoin. It is chemically synthesized drug and remains the most widely used drug in the treatment of malaria despite increase in parasite resistance. Chloroquine's reduced efficacy led to the development of synthetic analogue amodiaquine, mefloquine and halofantrine that are used to...
treat cases of uncomplicated malaria in areas, where chloroquine resistance is prevalent.

The second class of common antimalarials is folate antagonists, which includes the dihydrofolate reductase (DHFR) inhibitor pyrimethamine and proguanil and dihydropteroate synthetase (DHPS) inhibitors sulphamamide drugs, sulphadoxine and dapsone. These antifolates act synergistically against folate synthesis inhibiting parasitic pyrimidine, thus parasitic DNA. Sulphadoxine-pyrimethamine (SP) is the most important and widely used drug of this series.

The third class of antimalarials is based on the natural endoperoxide artemisinin, which was extracted from Chinese traditional medicine *Artemisinin annua*. The active constituents of the plant were identified and purified in the 1970s. Artemisinin does not have good solubility in oil and water, which led to the poor bioavailability and absorption of drug. The presence of stable endoperoxide bridge led to the development of various synthetic oil and water soluble derivatives, which includes dihydroartemisinin (DHA), arteether, arteether, artesunate and artelinic acid to improve pharmacological property to the parental drug artemisinin. This group of antimalarials is the most rapidly acting and effective against multi drug resistant strains of parasite. The artemisinin derivatives are also active against the sexual forms of the parasite (gametocytes) taken up by the mosquito and can therefore reduce transmission rates (Chen et al., 1994). The precise mode of action of artemisinin and its derivatives is unknown, however, it is generally proposed that the endoperoxide bridge is cleaved to generate free radicals, which are strong alkylating agents and forms covalent bond with various parasitic proteins, including membrane transporters (Eckstein-Ludwig et al., 2003). The Half life period of artemisinin is short thus leading to the problem of recrudescence. WHO has banned the monotherapy of artemisinin, thus there is an ardent need to find out suitable candidate with artemisinin for its combination therapy (WHO, 2006).
The rising problem of resistance to the classical drugs (chloroquine and sulphadoxine/pyrimethamine) and the problem of recrudescence of artemisinin, stresses the need to look for new antimalarial agents. Moreover, the most effective and affordable antimalarial drugs are either costly or inflict severe side effects. Thus it becomes important to look for newer antimalarial strategies to combat the problem of malaria. Mankind has always mined plants for the treatment of various ailments. The most important antimalarial drugs (Quinine and Artemisinin) have been obtained from Cinchona sp. and Artemisia annua plants respectively. The success of these drugs has broadened the search for natural plant products as a source of novel drugs for malaria.

**Traditional Medicine**

Traditional medicine serves as an important source for the treatment and development of newer drugs for various diseases. Herbal medicines constitute a major share of health system worldwide. World Health Organization (2006) has estimated that eighty percent of the world’s population use herbal medicine for their primary health care. Furthermore, herbal medicine is expected to be inexpensive and with out any severe side effects. The search for new drugs through the evaluation and validation of traditional medicines offers a good opportunity and a highly credible channel for the discovery and development of better medicines. The Research Initiative on Traditional Antimalarial Methods (RITAM) was established with aims and objectives to develop a strategy for more effective, evidence based use of traditional medicines that could contribute to malaria treatment (Willcox and Bodeker, 2000). Plants contain various secondary metabolites, which help in the treatment of various diseases. The investigation of traditional medicinal plants from various countries has led to the discovery of large number of antimalarial compounds with significant structural variety (Leone et al., 2007). In India, more than 70 percent population still use
alternative system of medicine (Vaidya and Devasagayam, 2007). Recently developed antimalarial Ayush-64, is a combination of four plants namely Alstonia scholoris, Picrorhiza kurroa royle, Swertia chirata and Caesalpinia crista (Kazim et al., 1991), is a potent example of herbal therapy. Xanthium strumarium, Ajuga bracteosa and Berberis aristata are known for their antimalarial properties in traditional medicine. However, their clinical efficacy and therapeutic role has not been established yet. Thus, it would be worthwhile to put field practice to laboratory investigation in order to provide relevant scientific evidences for their use and quest for newer drug discovery.

Homeopathy is an alternative science based upon stimulating body’s defense system instead of attacking pathogen directly. Homeopathy offers protection with out undue side effects and has been in use for the treatment of various diseases. At present, there is no large scale research evidence to support the use of homeopathy in preventing malaria, however, historical use of homeopathic medicine to strengthen individual immune system is well documented (Khuda-Buksh, 2003). The history of homeopathic treatment is based upon Cinchona testing of malaria treatment by Dr Samuel Hahnemann. The bark of Cinchona is a source of variety of alkaloids and the best remedies in chronic liver troubles, anemia and intermittent fever (Kleijnen et al., 1991). Kent (1974) have listed 45 homeopathic remedies for malaria among which Arsenicum, Bryonia, Cinchona, Ipecacunha, Eucalyptus globus, Natrium muriaticum, Vertrum album, Pulsatilla, Rhus toxicondendron, Nux vomica, sulphur are most frequently used for the treatment of disease. Eupatorium perfoliatum and Arsenicum album have been found to inhibit \textit{P. berghei} infection effectively in BALB/c mice (Lira-Salazar et al., 2006).

Search in traditional medicinal plants and homeopathy may provide safe, inexpensive, new antimalarial components, which is a dream of malaria control strategy. Keeping this in view the present study has been undertaken with following aims and objectives:-
• Evaluation of antiplasmodial efficacy of Ajuga bracteosa, Xanthium strumarium and Berberis aristata on schizont maturation inhibition of P. berghei in vitro.

• Phytochemical screening and evaluation of acute toxicity of plant extracts.

• Evaluation of suppressive, preventive and curative activities of traditionally used antimalarial plants, Ajuga bracteosa, Xanthium strumarium and Berberis aristata in vivo.

• In vivo evaluation of antiplasmodial efficacy of homeopathic medicines, China and Eucalyptus to clear P. berghei infection in BALB/c mice.

• Comparative study of effective concentrations of extracts, homeopathic medicines with quinine and artemisinin to clear P. berghei infection in BALB/c mice.

• Scanning electron microscopy of red blood cells and white blood cells of various groups treated with extract/drug/homeopathic medicines.

• Histological study of liver, spleen and kidney of mice, treated with various concentrations of extract/drug/homeopathic medicines.

• Evaluation of effect of various analogues on the enzyme activity of tissues (liver and spleen) of various treated groups.