Chapter II

Malaria and Amoebiasis
2.1.1 THE THAR DESERT ECOSYSTEM - MALARIA IN RAJASTHAN

The Indian Thar desert is located on the south-eastern fringes of the great Palaearctic desert, extending over two continents, from Sahara to Gobi desert (Prakash 1997) and occupies 12% area of the country with one of the most densely populated districts with no less than 12.8 million people (Joseph 1997). It is a sandy plain in the west and in the north west of the Aravalli axis covering a major part of Rajasthan apart from Gujarat, Punjab and Haryana (Sharma 1995). The Great Indian Thar Desert lies approximately between 24°-30° latitude North and 68°-78° longitude East. The Thar Desert has extremes of ambient temperature, below -0° to 50° C. The annual average precipitation, which until recently was about 250 mm., has since 1990 been about 350-400 mm. (Tyagi 1995) (Plate1)

The Thar Desert is a highly complex ecosystem, which is under the constant stress of change. Due to its xeric character, the Thar Desert in Rajasthan has always been regarded as an area hypo endemic for malaria. However, the scenario of both disease, and morbidity & mortality seems to have altered a great deal during the past decade. This is attributable to ecological and physiographical changes in the Thar region mainly due to the construction of three major canal systems in the desert, the Ganga canal, Bhakra-Sirhind Feeder canal and Indira Gandhi canal (Plate 2). The history of malaria in the Thar Desert indicates that until the early eighties, malaria prevalence in any desert district had seldom taken form of an epidemic, irrespective of the excessive rains during many a year since 1901. However, because of the application of "green revolution" technology and policy, the Thar has experienced several outbreaks of malaria, mainly due to P. falciparum, claiming several hundred lives during 1994 in Jaisalmer with choloquine resistant strains being reported (Sharma et. al. 1996). Also during late 1992 and early 1993 an epidemic broke out simultaneously in several districts in Bikaner, Bajju and Kolayat with many cases of cerebral malaria. (Jain 1994)

More than 2000 species of vascular plants play a vital role directly or indirectly in the economy and subsistence of about 42 lakhs tribal population of the State, Rajasthan.
Plate 1  Lengthening evening shadows in the desert sands of a harsh and arid, pristine environment. A small cluster of a lonely settlement separated and unconnected by basic minimum civic amenities, many of which can be seen from the road-side in the distance, scattered at the edge of the desert in Bikaner district.

Plate 2  One of the three major canal systems invading the Thar desert- The Indira Gandhi Canal under the bridge on the road to Bajjiu from Bikaner. The dramatic increase in vegetation and development has decreased the desert significantly.
2.1.2 PANDEMIC PREVALEANCE OF MALARIA

The word malaria was derived from two Italian words, i.e., "mala" and "aria" meaning "bad air" also known as Roman fever. The world has been plagued by malaria for some 3500 years of recorded history (Spracklen and Whittaker 1984). Malaria still remains one of the greatest causes of illness and death in the world (Choudhuri 1996). Each year this disease infects up to half a billion people and exacts a toll of roughly 2 million deaths, primarily of children under 5 years of age (Olliaro et. al. 1996). The emergence and spread of multidrug resistant malarial parasite esp. P. falciparum strains being resistant to many antimalarial drugs have hampered the eradication of the disease. WHO experts say that the number of people worldwide infected with malaria is still increasing at the rate of about 5% annually despite the extensive programme by the drug control and the eradication by the WHO (Dutta 1995). The government of India has been spending 30 - 35% health budget (Rs. 120 - 140 crores annually) on malaria control alone and an equal amount is shared by the state governments, except by the north eastern states where it is 100% centrally sponsored scheme (Sharma 1998).

In 1978 at Alma Ata conference World Health Assembly, emphasised the need for malaria control to be integrated with primary health care by the help of chemotherapy and alternative drugs development and development of vaccines specific to sporozoites, asexual erythrocytes and gamete related exoerythrocytic stage immunisation (Bruce-Chwatt 1985).

If we take as our standard of importance the greatest harm to the greatest number, then there is no question that malaria is the most important of infectious diseases — Sir Macfarlane Burnet.

It has an even greater impact on the disability-adjusted life years (DALYs) to estimate the disease burden for purposes of evaluating the cost effectiveness of interventions (Olliaro1996).

2.2.1 LIFE CYCLE OF PLASMODIUM FALCIPARUM

The causative agent is an unicellular organism belonging to the Phylum Protozoa, Subphylum Sporozoa and Genus Plasmodium. Although there are four Plasmodium species namely P. falciparum, P. vivax, P. malarie and P. ovale that affect humans, most of the mortality, an estimated 1,000,000 to 2,000,000 deaths per year,
is attributed to *P. falciparum*, the most virulent of the four species for the fatal tertian malignant malaria (Meshnick 1997). The malarial parasite, *P. falciparum* passes its life cycle in two different hosts and in four different stages as shown in Fig.1.

2.2.2 PLASMODIUM AND THE ERYTHROCYTE-IN VITRO

The invasion of erythrocytes by malaria parasites causes major changes in the structure, composition and function of the host cell membrane. During its intra-erythrocytic development, (stages of rings, trophozoites and schizonts as shown in the plates 3,4 and 5) the parasite imports exogenous nutrients and exports proteins and lipids into the host cell cytoplasm (Goodyer et. al. 1997). The rate of hemoglobin catabolism is highest at the trophozoite stage. Heme polymerase is located in the acidic vacuole of the parasite and uses FP (Ferriprotoorophyrin IX) that has been released from hemoglobin as substrate for biosynthesis of hemozoin (Orjih 1997). During its development, the parasite modifies the erythrocyte plasma membrane, mainly to allow for the entry of the nutrients and to dispose off waste products resulting in setting up new permeability pathways, which are essential for its survival (Gumila et. al. 1997).

*In vitro* testing is an important preliminary to *in vivo* screening and has many scientific advantages besides the cost and time required for *in vivo* tests but suffers from the drawback that they are not necessarily predictive of *in vivo* testing. Such *in vitro* studies can be used to study parallel *in vitro* cytotoxicity tests for its cytological studies, particularly with the changes in erythrocyte. The red cell membrane is perhaps the most complex natural membrane and one of the simple methods of analysis of disruptive effects of substances on membrane is based on the rupture of erythrocyte i.e., rate of *in vitro* hemolysis (Jaurand et. al. 1980). Drug induced hemolysis can be studied by noting different effects on the rate of its extent of hemolysis. Cytotoxicity can be thus studied at low concentrations to establish the relationships between hemolytic activity by membrane externalization and internalization by the drug along with the parasite (Sheetz and Singer 1974).

2.2.3 RESISTANT AND SUSCEPTIBLE STRAINS

The confirmation of chloroquine resistant asexual parasites became a challenging task in the identification of the various strains and the mechanisms they adopt to avoid the action of antimalarials in general and in geographic regions in particular. This resistance has led to recrudescent infections and an ever increasing
Fig. 1: Life-cycle of *P. falciparum* (Courtesy, Malaria Research Centre, Delhi)
Plate 3  Photomicrograph showing matured rings of *P. falciparum in vitro* (2000 x)

Plate 4  Photomicrograph showing matured trophozoites of *P. falciparum in vitro* (2000 x)
Plate 5  Photomicrograph showing two schizonts in a single erythrocyte and another with a ring, of *P. falciparum* *in vitro* (2000 x)
survival advantage and a greatly increased reservoir of infection reinforced by discovery of an ever-increasing number of strains. Plasmodium parasites isolated from patients inherently demonstrate genetic heterogeneity and it is therefore difficult to identify the genes that affect the level of chloroquine resistance. DNA amplification has been shown to occur in response to increasing doses of chloroquine and is lost upon removal of the chloroquine. Various combinations of synthetic drugs with natural products may by pass this resistance in some way. (Lim and Cowman 1996). Cross resistance to other quinine derived drugs is also well known to occur, even as co-resistance too becomes an important mechanism which can be tested in vitro, to exclude the possibility of natural resistance. Pharmacodynamic factors have been shown to reduce the parasite biomass by factors of $10^4$ as shown by a single dose of artemisinin (Mockenhaupt 1995).

A great deal of attention has been focused on the need for attenuating the effect of chloroquine which inspite of still being the premier antimalarial has been outwitted by the Plasmodia in developing resistance. The development of modulators of chloroquine action have been researched and studied extensively. These can potentiate the action of chloroquine and have the advantage of reducing its ultimate dosage and side effects even in resistant strains. Traditional uses of locally available crude plant formulations have had a rich folklore for believing that these can now be used in combination with chloroquine as a routine.

Therefore there has been a rampant proliferation of sensitive and resistant strains of \textit{P. falciparum}, each of which responds differently to standard antimalarials. 95 % active falciparum malaria cases were found to be resistant to chloroquine during the 1994 epidemic in Rajasthan and were related to a change in immune status of the human host (Sharma et. al. 1996). The non-immune and semi immune states of such infectivity depend largely on the effective plasma concentration of CQ that can be achieved without serious side-effects (Basco and Bras 1990). Chemosensitizers and potentiaters of chloroquine action may well help in reducing the dosage of chloroquine to prevent toxic effects in humans and enlighten the advantages of polychemotherapy including the use of crude plant/herbal products.

\textit{In vitro} studies of chloroquine potentiation and resistance reversal can be shown due to the following:
i) Direct action on the parasite, by either the greater accumulation of the drug in the parasite food vacuole by calcium antagonists (Platel et. al. 1998), or an increase in the steady state Chloroquine accumulation with a rise in the pH gradient (Knowles et. al. 1984); 

ii) Other cytoplasmic host-parasite interactions (Olliaro et. al. 1989); 

iii) Cross resistance between related drugs similar to multidrug resistance in tumor cells e.g. with Verapamil (Martin et. al.1987); 

iv) By unrelated drugs like antihistaminics (Basco et. al. 1991), anti viral amatidine (Evans and Havlik 1993) and other newer drugs (De et. al. 1993) and finally; 

v) The emergence of the parasitic phenotypes which may not be mechanistically linked to multi-drug resistance (Dieckmann-Schuppert et. al. 1993), but have a molecular basis of some genetic mechanism in the Plasmodium.

Not all strains of Plasmodium show common phenomenon associated with cross resistance to similar drugs which suggests the intriguing possibility that drugs can be designed with selective activity against individually CQ resistant strains (Geary et. al. 1987). In short time incubations, verapamil was found to increase net chloroquine accumulation in erythrocytes infected with both chloroquine-sensitive and resistant organisms, but only to affect the chloroquine susceptibility of the latter. Verapamil is the best studied drug for chloroquine reversal and by changing the external medium pH, this property of chloroquine was used to alter the cytotoxicity phenotype of genetically chloroquine-sensitive and resistant trophozoites. (Martiney et. al. 1995).

Plant extracts from a series of ethnobotanically identified plants which have been well evaluated for their chloroquine potentiating action have helped in establishing the methodology of deriving the dose response isobolograms of combinations with chloroquine and their fractional effects on synergistic, additive and antagonistic effects (Ratsimamanga-Urverg et. al. 1994a). Extraction of plant materials followed by fractionation and further fractionations showed better potentiating activity with “Hazomalamy “ identified as Hernadia voyronii (Ratsimammanga-Urverg et. al. 1994b).
2.3 LIFE CYCLE OF ENTAMOEBA HISTOLYTICA

*Entamoeba histolytica*, which has a worldwide distribution, is an enteric protozoan that exists in either trophozoite or cyst form (Ruiz-Palacios 1997). It belongs to the pseudopod-forming protozoan superclass Rhizopoda within the subphylum Sarcodina in the family Entamoebidae, order Amoebida and Class Lobosa. The trophozoite form of *E. histolytica* was described by Losch in 1875 from organisms found in a patient with chronic dysentery. Councilman and LaFleur described the clinical evidence of the association of the organism with dysentery in 1891. Quincke and Roos described the cyst form in 1893, and Fritz Schaudinn named the organism *Entamoeba histolytica* in 1903 (Ravdin and Petri 1995).

The life cycle is shown in Fig. 2. It is relatively simple and includes cyst and trophozoite stages. The trophozoites are found in the intestinal tract of the host where they feed on bacteria and cellular debris except when they become invasive (Gillin & Diamond 1981, Kraoul et. al. 1997). The encystment of the trophozoites is stimulated by unidentified factors in the fecal stream. After the cysts are ingested, no changes occur in an acid environment, but when the pH becomes neutral or slightly alkaline, the encysted organism becomes active with the outcome being the emergence of small trophozoites which develop into normal trophozoites when established in the large intestine, invade colonic mucosa causing amoebic dysentery (Mukherjee 1966). Cyst formation occurs only within the intestinal tract.

The trophozoites release enzymes or toxic products, which disrupt the intestinal barrier by adhering to the intestinal mucosa. Hematogenous transport of the trophozoites via the portal vein follows, and the trophozoites destroy the liver cells resulting in the formation of an amebic abscess (Fujihara et. al. 1996). Although extra intestinal amebiasis is most severe, intestinal amoebiasis is the primary and most frequent form of amebic infection & disease (Shibayama et. al. 1997).

2.4 DRUG RESISTANCE AND SIDE EFFECTS

The problem of CQ resistant falciparum malaria first described in Colombia in 1961 is ever increasing. In Africa first cases were reported in 1978 from Kenya and Tanzania, subsequently from other African countries (Blumenfeld et. al. 1984, Handunnetti et. al. 1996, Deacon et. al. 1994, Isaacson et. al. 1984, Schapira et. al. 1993). The first case of Mefloquine resistance was reported in Thailand in 1982 (Mockenhaupt, 1995). Historically drug resistance has occurred only in *P. falciparum*.
Fig. 2: Life-Cycle of *E. histolytica* with tissue phase (invasive forms) outside the normal circle. (Courtesy, *Manson's Tropical diseases*, Ed., Manson-Bahr and FIC Apted, Bailliere Tindall, London 1983).
infections however resistance to CQ is now also reported in *P. vivax* (Olliaro et. al. 1996). In India the first CQ resistance was reported in Assam in 1973 (Sehgal et. al. 1973).

The side effects of Chloroquine is associated with psychosis, personality change, depression & delirium (Good and Shader 1977), hypoglycemia (Davis 1997), skin depigmentation (Selvaag 1996a, Selvaag 1996b), retinal toxicity (Olansky 1982), nausea & vomiting, ringing in ears, hearing loss, dizziness, headache and disturbed vision (Meshnick 1997). Quinine shows abscesses and hypoglycaemia as side effects (Wise 1996). Therefore alternative more potent antimalarial drugs like xanthones (Ignatushchenko et. al. 1997) alone and in combination are needed (Karbwang et. al. 1992). Thus, with the problem of resistance on one hand and multitude of side effects on the other, it became inevitable to look for an alternative drug that would cure for this deadly disease.

Adverse side effects of CQ and emergence of CQ resistant strains of *P. falciparum* have necessitated the urgency of the search for novel, well tolerated and cost effective agents to treat CQ-resistant malaria (Radloff 1996, Anderson et. al. 1991, Anderson et. al. 1995, Clark et. al. 1983).

Antiamoebic drug, Metronidazole was shown to possess mutagenic activity (Connor et. al. 1977). It was also shown to induce lung tumors and increase incidence of malignant lymphomas (Rustia and Shubik 1972). In addition to the possibility of developing resistance to the drug through widespread and unnecessary use, metronidazole is intended only for elimination of tissue-invasive organisms and is not very effective against intestinal lumen infections (Clark 1998). Emetine and Cephaeiline are known for their toxicity as involving primarily gastrointestinal, cardiovascular and neuromuscular focii (Manno and Manno 1977).