INTRODUCTION
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Malaria parasites are with us since the dawn of time. They probably originated in Africa along with mankind and fossils of mosquitoes up to 30 million years old show that the vector for malaria was present well before the earliest history.

The first recorded description of malaria and its treatment dates back to 1600 B.C. in Edwin Smith Surgical Papyrus of ancient Egypt indicating that bitter bark of the cinchona tree was used by Peruvian Indians (Breasted, 1930). Later in 400 B.C., Hippocrates described the manifestations of the disease relating them to time of the year and habitat of the patients (Boyd, 1949).

The term 'Malaria' has been derived from two Italian words - 'mala' meaning 'bad' or 'foul' and 'aria' meaning 'air'. This terminology was based on the misconception that malaria was caused by the foul air coming from the marshes. Although malaria parasite was discovered by Laveran in 1880 and its life cycle in mosquito elucidated by Sir Ronald Ross in 1898, yet the terminology has not changed till date.

Malaria is considered an impediment to economic progress and hence, a disease of poor nations. Economists have found that it is not the poverty that determines malaria risk but the prevalence of malaria has major impact on the country's economy (Gallup and Sachs, 1998).

Malaria caused by Plasmodium, is by far the most deadly and devastating parasitic disease in the world. It kills one person often a child
under five – every 12 seconds (Butler et al., 1997). World Health Organization (WHO) has estimated that over 300 million cases of malaria occur annually, out of which 1.1 million people die because of this disease. These records, although shocking, are probably underestimates in light of the fact that only a fraction of all the malaria cases are reported annually and deaths among children are often attributed to other childhood illnesses (WHO, 1999a). In Africa alone, 28 million reported cases of malaria are believed to represent only 5 – 10% of the total malaria incidences on the continent (Hamoudi and Sachs, 1999). The global burden of malaria due to *P. vivax* has been estimated to be 70.80 million cases annually. Of these, 10 – 20% cases occur in Africa. Outside Africa, *P. vivax* accounts for 50% of all the malaria cases (Mendis et al., 2001). Although reported vivax infections through childhood and adult life are rarely lethal, yet they have deleterious effects on the physical, mental, social and economical well-being of the individual.

The groups which are at risk include pregnant women, children especially below the age of 5, travelers and soldiers etc. Ever-increasing intercontinental travelling, trading and migration mean that malaria eradication cannot be taken for granted. Italy, which was declared malaria-free officially by WHO in 1970, reported an imported case of *P. vivax* malaria in 1997 (Baldari et al., 1998). Airport malaria is contracted by the westerners who travel through malarious countries and wait on planes that are being refueled in malarious areas. Soldiers also are at very high risk. Malaria was the most common cause of hospitalization among American troops deployed in Somalia.
Government of India launched National Malaria Control Programme (NMCP) in April 1953. The strategy was shifted from 'control' to 'eradication' of malaria with formation of National Malaria Eradication Programme (NMEP) in 1958. However, the scheme was again modified in 1977 and the Modified Plan of Operation (MPO) was introduced with the aims of preventing/reducing the morbidity and mortality caused by malaria. MPO was considered successful as the number of malaria cases was reduced by 1984 and was maintained as such for about a decade. However, resurgence of malaria was reported in 1994. 29 worst-affected cities/towns were identified which contributed to over 80% of the urban malaria cases.

The number of malaria positive cases in India increased from 21.26 lakhs in 1992 to 30.36 lakhs in 1996. In 1997, the number reduced to 25.52 lakhs which was 18.9% less as compared with the preceding year. In 1997, six states namely Andhra Pradesh, Arunachal Pradesh, Haryana, Orissa, Rajasthan and Tripura were identified as epidemic-prone/worst-affected by Expert Committee. According to WHO report of 1998, there were 21.9 million estimated cases of malaria in South East Region in 1995 and India accounted for 85% of them (Comptroller Auditor General of India, 1999). In the same report, WHO claimed the actual number of malaria cases to be six times higher than the reported cases.

The high incidence rate of malaria is due to the nature of the parasite and the vector. Not only is the parasite highly complex, but the mosquito also is a sexually reproducing organism capable of mixing genes during reproduction. As a result mosquitoes quickly evolve to acquire insecticide resistance. It is also believed that the malaria parasite co-evolved with the
human species, hence the two organisms are probably well adapted to one another (Hamoudi, 2000).

Other reasons of disease spreading include changes in land use linked to activities like road building, mining, logging, agricultural and irrigation projects and changes in the climate like global warming and El Nino effects.

The malaria pathogen is a parasitic protozoan, *Plasmodium*, four species of which infect the humans: *falciparum*, *vivax*, *malariae* and *ovale*. These different species cause somewhat different types of malaria, the worst being caused by *P. falciparum*. The complications caused by falciparum malaria include 'cerebral malaria' in which brain is infected, 'severe malaria' in which the infection gets out of control and 'placental malaria' causing grave complications during pregnancy. Unfortunately, this type of malaria is frequently resistant to drugs. The other species cause a debilitating illness characterized by spells of chills, fever and weakness which is rarely fatal. Malaria caused by *P. vivax* and *P. malariae* is relapsing if not properly treated.

Malaria parasite has a complex life cycle requiring both vertebrate and invertebrate hosts (mosquito) for its survival and propagation. The mosquito host cannot be any, it has to be the females of *Anopheles* species. Man being the intermediate host harbours two phases of development of parasite — exoerythrocytic schizogony occurring in hepatocytes and erythrocytic schizogony in erythrocytes. Within the human host, the life cycle initiates by the injection of sporozoites by bite of infected mosquito. However, the sporozoites are not injected directly into the bloodstream as depicted in many life cycle diagrams. By now, several studies have indicated that during
mosquito probing, transmitted sporozoites are deposited as clumps under the skin and they take significant time moving away from the site of mosquito bite (Ponnudurai et al., 1991; Sidjanski and Vanderberg, 1997). From the site of deposition, they reach hepatocytes either via efferent arteries or lymphatics. The parasite propagates in hepatocytes and is released as merozoites. These merozoites follow two courses. Some of them re-infect the hepatocytes and continue the exoerythrocytic cycle while the others enter the erythrocytes to initiate the erythrocytic cycle. Within erythrocytes merozoites pass through ring, trophozoite and multinucleated schizont stages. Free merozoites are liberated into blood plasma to re-invade the new erythrocytes. Clinical symptoms of malaria are due to this erythrocytic cycle of the parasite.

Some merozoites change into sexual forms which are taken up by the female Anopheles mosquito along with its blood meal. The sexual cycle is completed in mosquito gut through zygote, ookinete and oocyst stages and finally resulting in mature sporozoites. These sporozoites travel up the gut into salivary glands of the host and are introduced into the new vertebrate host during mosquito bite to reinitiate the asexual phase.

Several bio-control devices have been employed to combat the vector. Two larvivorous fish, Poecilia reticulata and Gambusia affinis were used to control mosquito population (Murthy, 1998). Leaf extracts of Ocimum sanctum and Leucas aspera have been found to be highly toxic to mosquito larvae and exhibit highly deleterious effects on adult mosquitoes (Murugan and Jeyabalan, 1999).
The discovery of the insecticide DDT (Dichlorodiphenyl trichloroethane) in 1942 and its first use in Italy in 1944 made the idea of global eradication of malaria seem possible. During 1950s and 1960s the reliance on DDT reached its zenith when WHO launched a campaign to eradicate malaria from large parts of the world. Earlier results were very encouraging. In sub-Saharan Africa, where mosquitoes are most difficult to control, DDT spraying resulted in great reductions in malaria (Kouznetsov, 1977). In less than two decades, Sri Lanka's malaria burden reduced from 2.8 million cases and 7,300 deaths to 17 cases and no death. India achieved similar results and several countries fully eradicated malaria (WHO, 1999b). Unfortunately, this campaign received a major setback when overuse of DDT in agriculture bred DDT-resistant mosquitoes. Back in malaria's grip, Sri Lanka returned to half a million cases annually by 1969 (Attaran et al., 2000).

On 10th Dec. 2000, the delegates in Johannesburg, S. Africa approved a treaty allowing the restricted use, rather than ban of DDT, primarily in agricultural practices. This could be made possible by the long and successful campaign by Malaria Foundation International (MFI) and malaria project (MP). MFI has supported an eventual and not immediate ban on DDT saying that an effective and affordable replacement must be found before DDT is banned (Attaran, 2001).

In 1988, Peruvian scientists found a way to produce a natural, ecologically - friendly alternative to DDT. This is a bacterium, Bacillus thuringiensis var israelensis H–14 (Bti) which produces a toxin that kills mosquito larvae for as long as 45 days. Bti can be efficiently cultured in coconut. Wu et al. (1997) have reported that scientists have expressed the
combination of Bti genes into a type of cyanobacteria eaten up by mosquito larvae. The bacteria express the toxin and are capable of eradicating the total population of mosquito larvae. Since larvae are not allowed to develop into sexually reproducing adults by Bti toxin, mosquito resistance is not observed to the toxin (Basu, 2001). Recent studies have, however, shown that Bti is highly toxic to the larvae of endangered monarch butterflies, Danaus plexipus and can also have other effects on the ecosystem (Losey et al., 1999). The ultimate danger of insecticides, natural or manmade, is the selection of stronger and healthier adult mosquitoes.

Insecticide-impregnated bednets (IIBN) have proved to be effective in reducing mortality and morbidity, especially in children. TDR News (TDR, 1996) reported that trials in Africa reveal about 5 lakhs of children can be saved from malaria by the proper use of bednets. A meta-analysis of randomized controlled trials has revealed reduction in child mortality by 19% because of these bednets (Lengeler, 2001). Another recent study has associated IIBNs with a 27% increase in survival rate of children aged 1 month to 4 years (Schellenberg et al., 2001). Unlike DDT, these bednets are treated with biodegradable pyrethroids. These pyrethroids are derived from a naturally occurring substance pyrethrum, which is found in the flower Chrysanthemum cineraraefolium. These pyrethroids remain active for approximately 6 months after which the bednets can be retreated. Such measures are also helpful in case of pregnant women as they could not be given any treatment for malaria due to the fear of affecting the foetus.

Extensive research has been carried out in the field of vaccine development. Sporozoite stage antigen, circumsporozoite protein (CSP), has
been a candidate in most of the early vaccines which entered phase I/II clinical trials but were stopped due to the problems of low immunogenicity and efficacy. Only one candidate vaccine SPf66 by Manuel Pattarroyo having antigens from both merozoite and sporozoite stages has undergone extensive field trials in South America with efficacy ranging from 30-90%. However, results from subsequent trials in Africa and South-East Asia were not as promising (Facer and Tanner, 1997; Miller and Hoffman, 1998).

Many investigators around the world are starting DNA-based malaria vaccine development effort called Multi-stage DNA Vaccine Operation or 'MustDo'. During the next 3-4 years they aim to assess a 5-gene pre-erythrocytic stage vaccine (MustDo 5) and 15-gene pre-erythrocytic and erythrocytic vaccine (MustDo 15) as DNA vaccines alone and as the primers in prime-boost regimens (James and Miller, 2000).

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. In the absence of any effective vaccine, chemotherapy seems to be the only resolve in curing the disease.

Chloroquine, the first synthetic antimalarial drug introduced in 1934 as blood schizontocide is still the drug of choice. However, chloroquine-resistant strains of Plasmodium emerged after few years only. Several other antimalarials have come and disappeared from the scene because of the resistance the parasite develops against them. These include pamaquine (1925), mepacrine (1930), proguanil (1945), amodiaquine (1946), primaquine
In 1972, artemisinin was extracted from a plant *Artemisia annua* in China. Artemisinin and its derivatives have shown very promising results in the control of parasite. Very recently, a side effect of ptyalism has been observed in patients treated with β-arteether (Mishra and Mohanty, 1999). Combination drug therapy using tetracycline group of antibiotics with quinine (Watt *et al.*, 1992), mefloquine and artesunate (Looareesuwan *et al.*, 1994) have been found to be useful in treating multidrug-resistant *P. falciparum*. Curative blood schizontocidal activity was observed in *P. yoelli nigeriensis* and *P. cynomolgi* when their hosts were treated with the new macrolide antibiotic azithromycin (Puri and Singh, 2000). Other novel under-trial methods of preventing the disease include microtubule inhibitors which prevent the development and multiplication of *Plasmodium in vitro* and *in vivo*. These include colchicine, vinblastine and vincristine, taxol and taxotere. Their effects on microtubule inhibition varied from species to species.

Emergence and spread of antimalarial drug resistance results in the search of new drugs. Surolia and Surolia (2001) have described triclosan, an antibacterial agent found in mouthwashes, acne medicines and deodorants, as a promising new antimalarial agent. Triclosan acts as an inhibitor of fatty acid synthesis in *Plasmodium*, thus resulting in growth inhibition of the parasite *in vitro*.

The idea of malaria-transmitting mosquito as a harmless insect that does not transmit the disease is becoming more tangible year by year. This work of molecular entomology was started by TDR in 1991. The ultimate aim of this project is to replace the natural vectors of malaria in the wild with
transformed anopheline populations that are unable to spread the disease. The insertion of a gene for green fluorescence protein as a marker, into \textit{A. stephensi} marks the beginning of a new phase along the way to this goal (Catteruccia \textit{et al.}, 2000).

With the parasite becoming resistant to antimalarial drugs and the vector to insecticides and the non-availability of any potent malaria vaccine till date, the need to study the biochemistry of \textit{Plasmodium} is further emphasized. Lot of work is being done to study different metabolic pathways of the parasite so that novel potential antimalarial therapy (drug/vaccine) targets can be elucidated. Although all the enzymes of the glycolytic pathway have been studied in one or the other species of \textit{Plasmodium} yet only three enzymes lactate dehydrogenase, triosephosphate isomerase and aldolase have been studied in full detail whereby their crystal structures revealing new targets for antimalarial therapy have been completely elucidated (Dunn \textit{et al.}, 1996; Velanker \textit{et al.}, 1997; Kim \textit{et al.}, 1998). The present work has been undertaken with following aims in mind:

1. Detection of enzyme activity in normal and \textit{P. berghei} – infected blood and its fractions and cell-free parasite.
2. Localization of enzymes in subcellular fractions of \textit{P. berghei}.
3. Effects of different antimalarials on the activities of enzymes.
4. Purification of host enzymes.
5. Purification of parasite enzymes.
6. Characterization of purified parasite enzymes:
a. effect of pH

b. effect of temperature

c. effect of storage

d. effect of substrates
   - different substrates
   - different concentrations of a substrate ($K_m$ and $V_{max}$)

e. effect of specific activators of enzymes

f. effect of specific inhibitors including $K_i$ determination

7. Determination of molecular weights of the enzymes by SDS–PAGE.

8. Immunization of mice with different purified enzymes of parasite to raise antisera.