4. SCOPE AND PLAN OF WORK

4.1 Scope of Work

Paradoxically the rate of new drug development and new drug delivery in case of CM has been very low. The global response to this crisis has been inadequate. There are few new chemical entities in preclinical and clinical phases but these efforts could take considerable time to fructify. To overcome this problem, various approaches have been explored. One among them is the development of nanoparticle based drug delivery system which can enhance the therapeutic potency of existing drugs by improving their biopharmaceutics and pharmacokinetic property, reducing over drug toxicity and targeted delivery to the target organism or organ.

Pharmaceutical nanotechnology could provide unlimited opportunities for improving the efficacy of currently used anti-malarial drugs characterized by poor solubility, chemical instability, inadequate bioavailability profile and toxicity. Considering the nature of parasitic infections, it is expected that nanoparticles based drug delivery system will improve the specificity, tolerability, cellular uptake and protect the drug from extracellular degradation. The most important property of nanocarriers in the context of CM is the ability to target brain thereby improving the interaction with infected RBCs and their parasite membranes. Furthermore, long circulating nanosystems are able to improve the bioavailability of the drugs and reduce the doses employed in chemotherapy. So far, very limited attempts have been made of combining immunomodulator such as CUR with ARM for the effective management of CM.

ARM-CUR combination may prove superior from several perspectives. Both are from natural sources of long-term use, and as such, no resistance is known to CUR that is present in a dietary supplement. Both the drugs inhibit parasite SERCA pump (PfATP6). CUR can effectively control continued cytoadherence that persists long after parasite has been killed by inhibiting expression of various cell surface adhesion molecules (Hughes et al., 2010). Interestingly, CUR in addition to having a direct killing effect as an anti-malarial can also effectively control the non-parasite events like neuroinflammation and oxidative stress and thereby prevent cognitive deficits. Thus, this combination has a unique potential to prevent parasite recurdescence and relapse (Vathsala et al., 2012). Furthermore, CUR has been proposed as an attractive partner drug for ARM, due to its short half-life (1-2h), closely matching that of ARM; according to a resistance prevention perspective, the combination partner should have similar pharmacokinetic properties to provide optimum mutual protection. Treatment of CM, which is usually by IV injection, requires hospital admission. This represents an additional problem, since hospitals are
not easily and immediately accessible in all affected areas (Touitou et al., 2006). In the present study we propose a novel treatment approach- IN administration of anti-malarial drugs. The rationale behind using IN route for the treatment of CM is its convenience and non-invasive administration. Moreover, the nasal cavity may provide a port of direct entry for the drug to the brain. It is expected that the developed formulation will eventually lead to a better management of CM by controlling parasitic and non-parasitic events. The impact of nanomedicine on CM therapy will provide a new dimension to its effective control of morbidity.

4.2 Plan of Work

The project was carried out in the following stages:

Stage I: Preformulation studies
- Selection of drugs
- Analytical and bioanalytical method development by reverse phase high performance liquid chromatography
- Solubility and partition coefficient studies of drug
- Solubility and solution state stability of drug in simulated nasal fluid
- Compatibility study of drug and lipid by fourier transform infra-red spectroscopy and differential scanning calorimetry analysis

Stage II: Formulation studies
- Formulation and optimization of NLC using DoE approach
- Construction of pseudoternary phase diagrams for NE
- Formulation and optimization of NE using DoE approach

Stage III: Evaluation of lipid nanocarriers
- Particle/globule size, zeta potential
- Drug loading and entrapment efficiency
- Drug content, refractive index, % transmittance, viscosity, conductivity and pH.
- Thermodynamic stability
- Transmission electron microscopy
- Scanning electron microscopy
- Crystalline behavior and polymorphic modifications of drug and lipid by differential scanning calorimetry analysis and powder X-ray diffraction
- In vitro release by dialysis bag method
- Ex vivo diffusion by sheep nasal mucosa
- Release kinetics
Stage IV: Toxicity assessment
- *In vitro* cytotoxicity assay on SVG p12 cell line
- *In vitro* hemolytic toxicity on rat erythrocytes
- Nasal ciliotoxicity studies on sheep nasal mucosa

Stage V: Stability studies
- Stability Studies of NE and NLC at 4°C and 25°C for 6 months

Stage VI: Pharmacokinetic and brain uptake studies
- Pharmacokinetic studies of NLC in albino wistar rats

Stage VII: Pharmacodynamic studies
- Anti-malarial studies in *P. berghei* C57BL/6 female mice
- Histopathological analysis of brain, spleen and liver
- Evaluation of spleen size in mice