**ABSTRACT SUMMARY**

The present work reports the formulation development of a lipid nanocarrier system and surface engineered nanocarrier system for prolonged circulation and improved efficacy of a herbal oil (Annomaal), previously reported by our lab for antimalarial activity. Analytical method was developed to study the entrapment efficacy and release kinetics of the developed formulations. Physical characterization was done to confirm the surface modification of the engineered nanolipid carrier systems. The novel formulations were studied for antimalarial efficacy in *P. berghei* model using Peter’s four day test and its pharmacokinetics established in plasma and red blood cells.

**EXPERIMENTAL METHODS**

The experimental work included fractionation of seed extract into various components and the active herbal oil fraction, Annomaal, was identified.

**Analytical Method Development:** HPLC method was developed for quantification of Annomaal and validated as per the ICH guidelines.

**Formulation of Nanostructured lipid carrier system (NanoAnnomaal) and Surface Engineered lipid carrier system (P-NanoAnnomaal):** Nano lipid carrier system was fabricated for incorporation of Annomaal using high pressure homogenization technique. In brief, the herbal oil was dissolved in the lipid and lipophilic surfactant mixture at 70-75ºC. This lipid mixture was added to the hydrophilic surfactant mixture under constant stirring. The coarse emulsion was stirred at 70-75ºC for 30 minutes. The pre-emulsion was passed through High Pressure Homogenizer to obtain the Nano Lipid Carrier system (NanoAnnomaal). The method was optimized for product and process variables. The nanosystem was further surface modified using appropriate stealthing agent to obtain P-NanoAnnomaal.

**Characterization studies:**

**Drug content:** The formulations were evaluated for Drug content using the validated marker based HPLC Method.

**Physical characterization:** Particle Size and Surface properties were evaluated using PCS, TEM, SEM and AFM analysis.

**In vitro drug release kinetics:** In vitro drug release kinetics was tested using water shaker bath method at 37ºC at pH 7.4.

**Pharmacokinetic studies:** The formulations were tested for Plasma and RBC kinetics at 0.5,1,2,4,6,8,10,12,24 and 48h intervals using the validated HPLC method.

**INTRODUCTION**

Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected mosquitoes. In 2011, malaria caused an estimated 6,55,000 deaths mostly among African children. Resistance to artemisinin – a vital component of drugs used in the treatment of *P. falciparum* malaria – has been reported in a growing number of countries in South-East Asia. New antimalarials effective against parasite strains resistant to the antimalarial drugs used today are urgently needed. Apart from parasite resistance several of the currently available drugs suffer from additional drawbacks such as low efficacy, toxicity, and high-cost, accentuating the need for new antimalarial agents. Hence the challenge of the drug delivery is liberation of therapeutic agent to a specific target site at the right time in a safe and reproducible manner. As the blood stage infection is responsible for all symptoms and pathologies of malaria, Plasmodium infected RBC’s (pRBCs) are the main chemotherapeutic target (2).
In vivo efficacy studies: In vivo antimalarial efficacy was studied using Peter’s four day suppression test in *P. berghei* model.

Toxicity studies: Toxicological studies of the developed Lipid nanocarrier systems were carried out using acute and chronic toxicity studies as per the OECD guidelines. Genotoxicity studies were done using the Micronucleus assay and Chromosomal Aberration test.

RESULTS AND DISCUSSION

The herbal oil, Annomaal was formulated into Nano Lipid Carrier System (NanoAnnomaal) and further surface modified to obtain P-Annomaal. NanoAnnomaal showed mean particle size of 47nm, with PI of 0.19 while P–NanoAnnomaal showed a mean particle size of 63nm, and PI of 0.3. SEM, TEM and AFM analysis of the formulations confirmed the particle size, spherical shape and surface coating of the NanoAnnomaal.

![Figure 1: PCS results showing (a) NanoAnnomaal: 43nm (b) P- NanoAnnomaal : 63nm](image1)

Pharmacokinetic studies revealed that at the end of 48h, P–NanoAnnomaal demonstrated 6.6 times higher concentration in RBCs as compared to NanoAnnomaal, thereby proving the prolonged circulation and better efficacy of Surface modified nanoformulations. NanoAnnomaal showed reduction of dose to 50% of the original dose (250mg/kg) of Annomaal, when given intravenously. Acute and Chronic toxicity studies showed no significant changes in the blood serum parameters. Micronucleus assay and Chromosomal Aberration Assay showed that the developed novel formulations were found to be safe and non-mutagenic.

CONCLUSION:

The results show a novel herbal lead from a plant indigenous to the tropical regions against malaria. The drug has shown to be effective against *P. berghei*. Formulation of the herbal oil into nanocarrier system and its surface modification has resulted in prolonged circulation time and accumulation of the nanoparticulate system in the parasitized RBCs with significant reduction in dose and no significant toxicity, thereby opening perspectives for use in antimalarial therapy and further avenues for evaluation of this novel product at the clinical level.

REFERENCES:


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