Surface engineered Nanoannomaal: A nanoparticulate system for targeted antimalarial chemotherapy

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ABSTRACT SUMMARY
The present work reports the formulation development of a surface engineered lipid nanocarrier system for prolonged circulation and improved efficacy of herbal oil (Annomaal), for antimalarial activity. The antimalarial efficacy was tested in vitro using Trager Jensen continuous culture method and in vivo using Peter’s four day suppression test. The novel formulations showed good antimalarial efficacy with 50% dose reduction and prolonged activity in case of surface engineered nanoparticulate system.

INTRODUCTION
Malaria is a leading cause of deaths with an estimated 6,60,000 deaths mostly in children under 5 years of age, in 2012 (1). New antimalarial efficacies against parasite strains resistant to the antimalarial drugs in current use are urgently needed. Low efficacy, toxicity, and high-cost of the drugs, accentuate 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). The present work reports the formulation of surface engineered nano lipid carrier system of annomaal and in vitro and in vivo antimalarial efficacy of the same.

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

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 70-75°C. This lipid mixture was added to the hydrophilic surfactant mixture under constant stirring. The coarse emulsion was stirred at 70-75°C 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 engineered using appropriate stealth ing agent to obtain P-NanoAnnomaal (PNA).

Characterization studies: Particle Size and Surface properties were evaluated using PCS, TEM, SEM and AFM analysis.

Efficacy studies: In vitro antimalarial efficacy was studied using Trager Jensen continuous culture method using both the asynchronous culture with predominant ring stage parasites and synchronous trophozoite culture obtained using sorbitol treatment method. In vivo antimalarial efficacy was studied using Peter’s four day suppression test in P.berghei model. The surface engineered PNA system was tested at 100% (250 mg/kg) and 50% (125 mg/kg) dose levels and Artemether lipid nanocarrier system (ALN) was used as the standard drug.

RESULTS AND DISCUSSION
Formulation of Nanostructured lipid carrier system (NanoAnnomaal) and Surface Engineered lipid carrier system (P- NanoAnnomaal):

The herbal oil, Annomaal was formulated into Nano Lipid Carrier System (NanoAnnomaal/NA) and further surface engineered to obtain P-NanoAnnomaal (PNA). 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.

![Fig 1: PCS results showing (a) NanoAnnomaal: 43nm (b) P- NanoAnnomaal: 63nm](image-url)

(a)  
(b)
In vitro efficacy study:

With 24 h synchronous trophozoite late stage culture, std ALN showed antimalarial activity of 62.69 % at the end of 2 h indicating that ALN was highly effective at the late trophozoitic stage of the *P. falciparum* parasite. For asynchronous culture, Nanoannomaal (NA), showed 4.96 % antimalarial activity in 2 hours of incubation that increased to 39.59 % after 10 h. However the activity decreased to 30.48 % at the end of 24 h and further reduced to 22.86 % at the end of 48 h. With 24 h synchronous trophozoite late stage culture, NA showed antimalarial activity of 75.38 % at the end of 2 h indicating that NA was much more effective at the late trophozoitic stage of the *P. falciparum* parasite. At 50 % of therapeutic dose (125 mg/well), the activity of NA was found to be significantly reduced with maximum activity of 12.42 % seen at the end of 6 h. In case of PNA, for asynchronous culture, after 2 h of incubation, 9.61 % antimalarial activity was found which was approximately 1.93 times higher than the activity shown by NA at this time point, indicating higher and faster uptake of PNA by the infected RBCs. PNA showed time dependent increase in antimalarial activity, 73.82 % at 10 h, increasing to 88.88 % at the end of 24 h with the activity decreasing to 80.69 % at the end of 48 h. With 24 h synchronous late stage culture, PNA showed much higher activity (90.16 %) than NA (75.38 %) at the end of 2 h. Interestingly, PNA showed 79.48 and 75.23 % activity at the end of 24 h and 48 h at 50 % of the therapeutic dose. With PNA showing comparable antimalarial activity of at 100 and 50 % therapeutic dose, PEGylation of nanoparticles led to reducing annomaal dose by 50 %.

In vivo efficacy study:

From *in vivo* antimalarial efficacy studies it was evident that on 14th day post treatment, at 100 and 50 % of the therapeutic dose administered intravenously, PNA, showed at 72.23 % and 73.24 % antimalarial activity respectively. Complete cure of all the test animals was observed till day 30. Also by the end of 14th day std ALN showed 76.68 % antimalarial activity. Thus at the 50 % therapeutic dose level PNA showed equivalent antimalarial activity compared to 100 % PNA and the animals showed healthy recovery in 30 days. The results proved at 100 % therapeutic dose level PNA showed early onset of action and higher antimalarial activity compared to bare NA formulations (67.63 % activity) with sustained effect showing superior antimalarial activity.

CONCLUSION

The results show a novel herbal antimalarial lead from a plant indigenous to the tropical regions. The drug has shown to be effective against *P. berghei* and *P. falciparum*. Formulation of the herbal oil into surface engineered nanocarrier system has resulted in prolonged circulation time and accumulation of the nanoparticulate system in the parasitized RBCs leading to significant reduction in dose thereby opening perspectives for use in antimalarial therapy.

REFERENCES


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