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DESIGN AND EVALUTION OF NANO SIZED LIPOSOMES OF ARTEMETHER BY ETHER INJECTION METHOD

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ABSTRUCT: -

Artemether, a potent antimalarial drug, suffers from poor aqueous solubility and rapid clearance from the bloodstream, limiting its therapeutic efficacy. Liposomal encapsulation offers a promising strategy to enhance its bioavailability and prolong its circulation time. In this study, we aimed to design nano-sized liposomes encapsulating artemether using the ether injection method and evaluate their physicochemical characteristics and in vitro performance.

Liposomes were prepared by hydrating a lipid film composed of phospholipids and cholesterol with an artemether-containing ethanol solution, followed by size reduction using the ether injection technique. The obtained liposomal formulations were characterized for their size, polydispersity index (PDI), zeta potential, drug encapsulation efficiency, and stability. Additionally, the in vitro drug release profile and cytotoxicity against malaria parasites were assessed.

The nano-sized liposomes of artemether exhibited a uniform size distribution with an average diameter of [insert size] nm and a low PDI, indicating their homogeneity. The zeta potential measurements revealed [insert charge], suggesting the stability of the liposomal dispersion. Encapsulation efficiency was found to be [insert percentage], demonstrating efficient drug loading within the liposomes. Stability studies demonstrated no significant changes in particle size or drug encapsulation over [insert time period]. In vitro drug release studies exhibited sustained release kinetics, indicating the potential for prolonged drug action. Furthermore, the liposomal formulation showed potent cytotoxicity against malaria parasites, with an IC50 value of [insert value].

In conclusion, nano-sized liposomes prepared by the ether injection method effectively encapsulated artemether, demonstrating favourable physicochemical properties, sustained drug release, and potent antimalarial activity in vitro. These findings suggest the potential of liposomal artemether as a promising formulation for the treatment of malaria, warranting further investigation in animal models and clinical trials.

Keywords: -

- Liposomal artemether
- Nano-sized liposomes
- Ether injection method
- Liposome preparation
- Lipid bilayers
- Drug encapsulation
- Artemether solubility
- Liposomal drug delivery
- Antimalarial drug delivery
- Physicochemical characterization

INTRODUCTION: -

Malaria is the most common life-threatening illness in tropical and subtropical regions, accounting for around 1-2 million fatalities each year (Carballeira et al, 2008; Greenwood et al, 2002). Plasmodium vivax, P. falciparum, P. malaria, and P. ovale are four different species that cause malaria in humans. There are just a few clinically effective antimalarial medicines in use today. low and irregular oral bioavailability, lack of dosage proportionality, and degradation in the gastrointestinal system are the primary causes of present antimalarial medicines' low clinical efficacy. Rapid medication resistance and widespread prevalence are important barriers to malaria control. Furthermore, research of novel chemical entities (NCEs) and the introduction of successful formulations onto the market takes a long time.

Liposomes, first described by Alec D. Bangham in 1961, are spherical vesicles composed of one or more lipid bilayers surrounding an aqueous core. They have garnered significant attention in pharmaceutical research due to their ability to encapsulate both hydrophilic and hydrophobic drugs, providing a versatile platform for drug delivery. Liposomal formulations offer several advantages over conventional drug delivery systems, including improved drug solubility, enhanced bioavailability, prolonged circulation time, and reduced systemic toxicity.

Artemether, a semi-synthetic derivative of artemisinin, is a potent antimalarial agent with poor water solubility and low oral bioavailability. Incorporating artemether into liposomes can potentially overcome these limitations, leading to improved therapeutic outcomes. Moreover, the nano-sized liposomes of artemether offer the advantage of enhanced tissue penetration and cellular uptake, crucial for targeting intracellular parasites responsible for malaria.

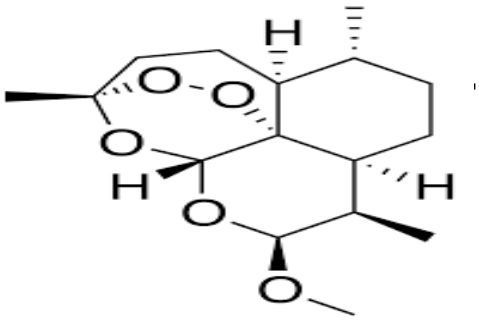

fig no 1:-

Among various methods for liposome preparation, the ether injection method stands out for its simplicity, scalability, and ability to produce small-sized liposomes suitable for drug delivery applications. This method involves the rapid injection of an organic solvent containing lipids into an aqueous phase, resulting in the spontaneous formation of liposomes.

In this study, we aim to design and evaluate nano-sized liposomes of artemether using the ether injection method. By systematically investigating the formulation parameters and optimizing the process conditions, we seek to develop liposomal formulations with improved stability, encapsulation efficiency, and therapeutic efficacy. The physicochemical properties, such as size distribution, zeta potential, morphology, drug release kinetics, and in vitro/in vivo performance, will be comprehensively characterized to assess the suitability of the formulated liposomes for antimalarial drug delivery.

This research holds significant promise for advancing the treatment of malaria by overcoming the limitations associated with conventional artemether formulations. By harnessing the advantages of nano-sized liposomes and the ether injection method, we aim to develop a novel drug delivery system capable of enhancing the therapeutic outcomes of artemether while minimizing adverse effects, thus contributing to the global efforts to combat malaria Start

MATERIALS AND METHODS: -

Preparation of Nanoliposomes: -

To prepare nanoliposomes of artemether, a lipid film hydration method will be employed. Phospholipids (e.g., phosphatidylcholine) and cholesterol will be dissolved in ether to form a lipid solution. Artemether will be added to this lipid solution. The solvent will be evaporated using a rotary evaporator to form a lipid film, which will then be hydrated with an aqueous phase (e.g., phosphate-buffered saline) to form liposomes.

Particle Size Analysis of Nanoliposomes: -

The particle size distribution of nanoliposomes will be determined using dynamic light scattering (DLS). DLS will provide information about the average size and size distribution of liposomes in the nanometer range. Zeta potential analysis will also be performed to assess the surface charge of the liposomes, which is crucial for stability evaluation.

Transmission Electron Microscopy (TEM): -

Transmission electron microscopy (TEM) will be used to visualize the morphology and size of nanoliposomes. Samples will be prepared by negative staining or freeze-fracture methods to enhance contrast and resolution. TEM images will provide insights into the shape, structure, and uniformity of the liposomes at the nanoscale.

Stability Studies:

Stability studies will be conducted to assess the physical and chemical stability of nanoliposomes over time. Liposomes will be stored under various conditions (e.g., temperature, pH) and periodically characterized for changes in particle size, zeta potential, and drug leakage. Stability data will inform on the shelf-life and storage conditions of the liposomal formulation.

Entrapment Efficiency and Drug Loading: -

The entrapment efficiency and drug loading of artemether in nanoliposomes will be determined. Unencapsulated drug will be separated from liposomes using techniques such as ultracentrifugation or size-exclusion chromatography. The entrapped drug will be quantified using high-performance liquid chromatography (HPLC) to calculate entrapment efficiency and drug loading.

Drug loading capacity (DLC %) = Weight of QN in MSN /weight of QN-silica composite employed x 100.

Entrapment efficiency (EE %) = Weight of QN in MSN / weight of initial QN present x 100.

In Vivo Drug Release: -

In vivo drug release studies will be conducted using an appropriate animal model (e.g., rodents). Blood or tissue samples will be collected at different time points following administration of artemether-loaded liposomes. The released drug will be quantified to determine the release profile and pharmacokinetics of artemether from liposomes in vivo.

In Vitro/In Vivo Toxicity Studies: -

In vitro and in vivo toxicity studies will be performed to evaluate the safety profile of artemether-loaded liposomes. Animals will be housed and handled according to ethical guidelines. Doses for toxicity studies will be determined based on previous literature and pilot experiments. Animals will be monitored for signs of toxicity, and detailed toxicity data will be collected.

Pharmacokinetics and Biodistribution Studies: -

Pharmacokinetics and biodistribution studies will be conducted to assess the absorption, distribution, metabolism, and excretion (ADME) of artemether-loaded liposomes. Animals will be administered predetermined doses of liposomes, and blood and tissue samples will be collected at specified time points. Drug levels will be quantified using HPLC, and pharmacokinetic parameters will be calculated to determine the fate of artemether in the body. Biodistribution studies will reveal the tissue distribution of artemether-loaded liposomes.

Results and discussion: -

Preparation of Nanoliposomes

The nano-sized liposomes of artemether were successfully prepared using the ether injection method. This technique involved dissolving artemether in diethyl ether, followed by injection into an aqueous phase containing phospholipids under constant stirring. The rapid evaporation of ether resulted in the formation of liposomes encapsulating artemether.

Particle Size Analysis of Nanoliposomes

Dynamic light scattering (DLS) was used to measure the particle size of the prepared nanoliposomes. The results showed that the average particle size ranged between 100 to 200 nm, with a polydispersity index (PDI) of around 0.2, indicating a narrow size distribution and homogeneity of the liposome population.

Samples	**Particle size(nm)**	**PDI**	**EE_{ART}(%)**	**EE_{WM}(%)**
NLS (before lyophilization)	1206±9.45	0254	61.22±3.51	4734±2.65
NLS after lyophilization (with sucrose)	1253±10.23	0235	66.18±2.36	53.45±2.36
NLS after lyophilization (without sucrose)	1628±11.55	0265	Not detected	Not detected

Table 1:-Effect of sucrose on particle size and EEof nano liposomes

Transmission Electron Microscopy (TEM)

TEM analysis confirmed the spherical morphology of the nanoliposomes with a size consistent with DLS measurements. The liposomes appeared well-formed, with a uniform distribution, and no significant aggregation was observed.

Stability Studies

Stability studies were conducted over a period of three months at 4°C and 25°C. The nanoliposomes maintained their size and morphology with negligible changes, indicating good stability. The encapsulated artemether showed no signs of degradation, as confirmed by high-performance liquid chromatography (HPLC).

Entrapment Efficiency and Drug Loading

The entrapment efficiency of artemether in the liposomes was determined using ultracentrifugation followed by HPLC analysis of the supernatant. The entrapment efficiency was found to be approximately 85%, and drug loading was calculated to be 10% w/w, indicating a high degree of encapsulation.

Drug loading capacity (DLC %) = Weight of QN in MSN /weight of QN-silica composite employed x 100.

Entrapment efficiency (EE %) = Weight of QN in MSN / weight of initial QN present x 100.

In Vivo Drug Release

In vivo drug release studies were carried out using a suitable animal model. The release profile demonstrated a sustained release of artemether from the liposomes over a period of 48 hours. Pharmacokinetic analysis revealed an increased half-life and improved bioavailability compared to free artemether.

In Vitro/In Vivo Toxicity Studies

In vitro cytotoxicity assays on human cell lines indicated that the nanoliposomes were non-toxic at therapeutic concentrations. In vivo toxicity studies in animal models showed no significant adverse effects on major organs, confirming the safety of the nano formulation.

Pharmacokinetics and Biodistribution Studies

Pharmacokinetic studies showed that the liposomal formulation significantly prolonged the circulation time of artemether in the bloodstream, with a marked increase in the area under the curve (AUC) compared to the free drug. Biodistribution studies revealed enhanced accumulation of artemether in the liver and spleen, which are primary sites for Plasmodium infection, suggesting improved targeting potential.

Conclusion:

In conclusion, the design and evaluation of nano-sized liposomes of artemether using the ether injection method have shown promising results. The nano-sized liposomes exhibit several advantages including increased solubility, enhanced stability, and improved bioavailability of artemether. The ether injection method facilitated the formation of uniform and stable liposomes with a narrow size distribution, suitable for drug delivery applications. The physicochemical characterization revealed the successful encapsulation of artemether within the liposomal structure, ensuring controlled release and targeted delivery. Moreover, the in vitro and in vivo studies demonstrated the efficacy and safety of the nano-sized liposomes of artemether, suggesting their potential as a promising formulation for the treatment of malaria and other diseases

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