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FORMULATION AND EVALUATION OF LIPOSOMAL GEL FROM CENTELLA ASIATICA FOR WOUND HEALING ACTIVITY: A REVIEW

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ABSTRACT

The present study aims to formulate and evaluate a liposomal gel containing Centella asiatica extract to enhance topical delivery and wound healing activity. Wound healing is a complex biological process involving inflammation, proliferation, and tissue remodeling. Centella asiatica is a well-known medicinal plant traditionally used for wound healing because of its active constituents such as asiaticoside, madecassoside, and asiatic acid, which promote collagen synthesis and angiogenesis. Liposomes were prepared by various methods like Thin- Flim hydration, Reverse phase Evaporation, Detergent removal, Solvent (Ether or Ethanol) Injection, Heating method, Microfluidic devices, and Sonication Method. Characterized for vesicle size, zeta potential, morphology, and entrapment efficiency. The optimized liposomal suspension was incorporated into a suitable gel base to obtain a liposomal gel formulation. The prepared liposomal gel was evaluated for physicochemical parameters such as pH, viscosity, spreadability, drug content, in-vitro drug release, and stability studies. The study concludes that liposomal gel of Centella asiatica is a promising and effective topical delivery system for wound healing applications.

KEYWORDS: Centella asiatica, Liposomes, Liposomal gel, Wound healing, Topical

formulation.

INTRODUCTION

The most innovative and successful drug delivery technique used in dermatology is liposomal gel-based formulation, which has several benefits over conventional topical medications. Dr. Alec D. Bangham developed the first liposomes in England in 1961 while examining blood coagulation and phospholipids. Liposomes are small particles getting an aqueous core surrounding by one or more external shells made of bilayered lipids. Their ability to increase skin penetration makes them effective instruments for treating a variety of dermatological disorders, regulate release profiles and improve the stability of medications. Because of their biocompatibility, similarity in structure to biological membrane liposomal formulation, and special capacity to encapsulate lipophilic and hydrophilic drugs while facilitating deeper skin penetration. These vesicles, which have a unique amphiphilic form with hydrophilic heads facing outward and hydrophobic tails pointing inward, are created when phospholipids in water self-assemble. Liposomes are usually made up of cholesterol and phospholipids, and their structure, composition, and proportions are almost the same as those of the host cell membrane.

Liposomes provide advantages over conventional dosage forms by significantly improving drug loading, drug delivery, and prolonged release. Liposomes have been demonstrated to be significantly better than traditional dosage forms, particularly for intravenous and topical administration, and have been widely used to improve the effectiveness of drug delivery through a variety of routes of administration.

Centella asiatica, more commonly referred to as gotu kola, It is an important medicinal plant widely used in Ayurveda, Siddha, and Traditional Chinese Medicine. It is widely recognized for its effectiveness in wound healing and skin health. Studies accomplished both in vitro and in vivo provide evidence that C. asiatica beneficially impacts a number of components associated with wound healing. Certain clinical trials have also demonstrated its beneficial effect on wound healing. Different varieties of C. asiatica have been applied orally or topically to patients with either acute or chronic wounds in these studies, particularly when applied topically.

Coagulation, inflammation, cytokine formation, cell migration, proliferation, and development are every component in the complicated biological process of wound

healing. Vascular development, extracellular matrix development and remodeling (including collagen production and deposition). The main elements of the extracellular matrix of the skin are type I and type III collagen. Both types of collagen are essential for the healing of wounds. Wound contraction and epithelial proliferation of cells occur. Numerous scientific studies have demonstrated that C. asiatica extracts, individual triterpene chemicals, and the combination of triterpenoids from C. asiatica enhance wound healing.

Despite Centella asiatica contains therapeutic potential, its quick metabolism, low bioavailability, and poor In pharmaceutical purposes, water solubility significantly limits the systemic absorption of its active substances. In order to overcome these barriers, modern formulation approaches such solid lipid nanoparticles, liposomes, phytosomes, nano emulsions, and polymeric hydrogels are being evaluated. These innovative drug delivery techniques improve the pharmacodynamic potential of C. asiatica therapeutics by enhancing solubility, stability, and controlled administration.

PLANT PROFILE

Botanical classification

The Apiaceae (Umbelliferae) family contains Centella asiatica. The plant develops in tropical and subtropical regions of Asia, including China, Indonesia, India, and Sri Lanka. It can be recognized by its characteristic fan-shaped leaves and tiny white to pinkish blossoms

Phytoconstituents

Centella asiatica's pharmacological actions are caused by triterpenoid saponins, specifically asiaticoside, madecassoside, and its aglycones Asiatic acid and madecassic acid. The plant also contains amino acids, volatile oils, sterols, polyphenols, flavonoids, and tannins.

Pharmacological Activities

Multiple pharmacological properties of Centella asiatica include anti-inflammatory, neuroprotective, hepatoprotective, wound-healing, antidiabetic, and anti-anxiety actions. Asiaticoside and madecassoside stimulate angiogenesis, fibroblast proliferation, and collagen synthesis, all of that support in tissue regeneration. The plant's neuroprotective properties stem from the modulation of neurotransmitters and the decrease of oxidative stress. In metabolic disorders, it lowers blood glucose and cholesterol levels while protecting the liver. Its antioxidant action, which combats reactive oxygen species, gives it anti-aging and skin-healing qualities. These various impacts enhance C. asiatica's reputation as a versatile therapeutic agent

that may both prevent and treat a wide range of illnesses.

Pharmaceutical Formulations

Creams and ointments, Liposomal gel, Capsules and tablets, Syrups and herbal teas, Cosmetic products (anti-aging creams).

CLASSIFICATION OF LIPOSOMES

1. Classification Based on Structure (Lamellarity)

According to the size and number of bilayer membranes (lamellarity) forming vesicles, liposomes can be divided into the following categories

- Unilamellar vesicles (ULV)
  - Small Unilamellar vesicles (SUV): 20–100 nm
  - Large Unilamellar Vesicles (LUV): 100–1000 nm
  - Giant Unilamellar Vesicles (GUV): >1000 nm
- Oligolamellar vesicles (OLV): 100-1000 nm.
- Multilamellar large vesicles (MLV): >500 nm**.**
- Multivesicular vesicles: >1000 nm.

2. Classification based on Composition and application

According to its composition and intended application, liposomes can be classified into many of different types.

- Conventional liposomes
- Long circulating liposomes or Stealth liposomes
- Cationic liposomes
- Targeted Liposomes
- Immunoliposomes
- Stimuli-responsive

3. Classification Based on Method of Preparation

- Thin film hydration (Hand shaking) method
- Sonication method
- Ethanol injection method
- Reverse phase evaporation method
- Detergent removal method
- Microfluidizatio

MATERIALS AND METHODS

❖ Materials

Dried aerial part of Centella asiatica,extracting solvents used methanol,ethanol(50% and 70%), water, chloroform,diethyl ether, No.1 Whatman filter paper,rota rod evaporator,hot air oven or tray dryer.

❖ Centella Asiatica leaf bioactive compound extraction

The Centella asiatica plant was thoroughly rinsed under running water. After being removed from the root and stem, the leaves were dried for four hours at 40±2 °C in a recirculating hot air drier until they reached a uniform weight. A mechanical crusher was then used to crush the leaves into a fine powder with an 80 mesh size. Powdered materials were xtracted at room temperature using a 1:23 (w/v) ratio of 62% ethanol in a Hielscher ultrasonic (UP50H). The transducer operates at a frequency of 30 kHz with a power rating of 50 W. The extracted material was centrifuged at 9000 rpm for 10 minutes at 4 °C. After straining the supernatant using No. 1 Whatman paper and evaporating the solvent at 40 °C under vacuum in a rotary evaporator, the remaining extract was collected, freeze-dried, and kept at -20 °C until required again.

METHOD OF PREPERATION OF LIPOSOMES

There are several methods that are used to prepare liposomes. The final characteristics of liposomes can be changed depending on the type of phospholipid and the manufacturing procedure. J. Y. Johnson developed the first artificial liposomes in the 1940s and patented the method for use in the pharmaceutical industry (I. G. Farbenindustrie Aktiengesellschaft). Several techniques can be used to prepare the liposomes.

Active loading, that occurs when drug entrapment is taking place after liposome formation, and passive loading, in which drug entrapment takes place right before liposome formation.

GENERAL INGREDIENTS

Natural and synthetic phospholipids, which include phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholline, phosphatidylserine, and phosphatidylinositol, are usually present in liposome composition. The two primary structural components that compose a significant portion of biological membranes are phosphatidylcholine, commonly referred to as lecithin, and phosphatidylethanolamine. Additional components like water, hydrophilic

polymer associated with lipids, and cholesterol may also be found in liposome bilayers.

Cholesterol has been employed significantly for improving liposomes' bilayer characteristics. It minimizes the permeability of water-soluble substances through the membrane and improves membrane fluidity and bilayer stability. The most significant advantage of liposomes is that their lipid membrane is composed of physiological lipids, which significantly reduces the risk of both acute and chronic toxicity.

PREPARATION OF METHODS

1. Method of thin-film hydration

Lipid thin films are deposited on the inside surface of a rotating evaporator flask using the Bangham method, a widely used technique in liposome manufacturing. This process involves dissolving lipids and hydrophobic substances in a suitable organic solvent inside a flask with a circular bottom. When the dispersion medium is added and stirred, heterogeneous liposomes are produced. The material that has to be encapsulated is either added to the lipid formation or combined with the buffer solution. Even with little concentrations of chemicals, the thin film hydration method is very repeatable; nonetheless, its encapsulation efficiency is poor.

2. Evaporation in Reverse Phase

This technique involves disintegrating the lipids in an organic solvent that promotes the development of inverted micelles, such as chloroform/methanol (2:1 v/v). A water-in-oil microemulsion is then produced by adding aqueous buffer. A rotary evaporator is then used to evaporate the organic solvent, forming a thick gel.

After then, the gel will collapse, producing liposomes. The liposomal gels demonstrated a regulated release with an excellent penetration profile, and the microemulsions' large aqueous center encourages the trapping of particularly hydrophilic molecules. The method uses a lot of organic solvent, and the solvent extraction procedure is laborious and slow.

3. Elimination of Detergent

In order to dissolve and hydrate phospholipids, this approach involves introducing a detergent, such as sodium cholate or alkyl glycoside, to the lipids. This prevents the hydrophobic parts of the lipids from interacting with the aqueous medium, creating micelles that include both the detergent and the lipid. The detergent is then gradually eliminated, enabling the development of lipid-rich micelles that naturally result in the production of

unilamellar vesicles.

Diluting the suspension with a buffer, which also increases the micellar size and polydispersity, is the simplest way to get rid of the detergent. However, because of the dilution stage, this method results in low liposomal concentration and poor EE of hydrophobic medicines. Alternatively, the detergent can be eliminated using the dialysis process. The detergent can also be removed using resin beads, centrifugation, and gel chromatography techniques.

4. Solvent (Ether or Ethanol) Injection Technique

The phospholipid is dissolved in ether during the ether injection process. The aqueous medium containing the intended substance to be encapsulated is gradually injected with a solution of phospholipids dissolved in ether. The mixture is heated to 55–65 °C to ensure adequate ether evaporation. SUVs and those made with ethanol injection have comparable characteristics. Ether may be effectively removed quickly because it evaporates at a lower temperature than ethanol, creating concentrated liposome solutions with comparatively high entrapment efficiency.

Employing this procedure, the phospholipid is dissolved in ethanol and an aqueous medium is made and boiled previously. A needle is used to quickly inject the ethanol solution containing the dissolved phospholipid into the aqueous medium that contains the item to be entrapped. To guarantee the development of liposomes, the mixture must be stirred at a high temperature (55–65°C). The ethanol will evaporate. The ethanol injection method is easy to use and may quickly create liposomes. Depending on the pace of ethanol injection, this technique can produce both large unilamellar vesicles (LUVs) and small unilamellar vesicles (SUVs). When the ethanol percentage is less than 7.5% of the total formulation volume, homogenous SUVs are created. Heterogeneous MLVs are created otherwise.

5. Sonication method

This technique transforms multilamellar vesicles into small unit layered vesicles. The MLVs receive the ultrasonic treatment. For getting the SUVs. Two different methods have been used. Methods of sonication: a) probe sonication; b) bath sonication. The probe is employed for dispersion, which is better appropriate for large quantities of diluted liquid but requires significant amounts of energy in a small volume (as well as a viscous aqueous phase or a high concentration of lipids). Despite the probe tip sonicator provides the liquid dispersion a high

energy input, the liposomal dispersion overheats and destroys lipids. Titanium is also released by the sonication tip into the liposome dispersion, which is then separated from the centrifugation before being utilized. Stainless steel attachments are the most frequently employed because of the previously mentioned reason. MLVs are sonicated by placement dispersion into the bath sonicator or introducing the probe sonicator's tip into the dispersion test tube. (5–10 minutes) The resulting dispersion is centrifuged after sonication, and the diagram indicates that the small MLVs and associated lipids will settle down although the SUVs will stay on top. The top layer is a pure dispersion of SUVs with different diameters since temperature, sonication, volume, composition and concentration, and sonication tuning all affect size.

CENTELLA ASIATICA LEAF EXTRACT LOADED LIPOSOMES

Composition of Centella asiatica liposomes.

S.NO	INGREDIENT	QUANTITY	ROLE
1	Soya lecithin	2-4%w/v	Lipid bilayer former
2	Cholesterol	0.5-1%w/v	Membrane Stabilizer
3	Centella asiatica extract	1-2%w/v	Active ingredient
4	Chloroform:methanol,2:1	q.s	Dissolves lipids
5	Phosphate buffer(6.8-7.4)	q.s	Hydration medium

- Leaf extract of Centella asiatica Soy lecithin and Cholesterol were incorporated in various ratios using methanol and chloroform to create loaded liposomes
- A rotary evaporator was utilized at 45°C to remove the organic solvent and create a thin lipid film.
- The lipid film was hydrated under orbital agitation using phosphate buffer solution (pH 7.4) with CALE (1 mg/ml).
- The suspension has been separated utilizing a homogenizer to create liposomes.

CHARACTERIZATION OF LIPOSOMES

1. Vesicles size and size distribution

One of the most important parameters in liposome characterisation is the particle size. Malvern Instruments' Zeta sizer (Dynamic Light Scattering) was used to determine the liposome's size. After diluting the dispersion with Millipore filtered water to the proper scattering intensity at 25°C, the sample was put in a cuvette of standard size.

2. Zeta potential

Zetameter was employed to determine the surface of charge drug-loaded vesicles. The mean percentage zeta potential and charge on the liposome was determined after a 60-second analysis period.

3. Drug Entrapment Efficiency

To separate the free medication, the liposome suspension was ultra-centrifuged for one hour at 5000 rpm. The supernatant contains free medicine near the centrifugation tube wall and liposomes in the suspended stage. After collecting the supernatant, it was centrifuged once again for 30 minutes at 5000 rpm. The result was a homogeneous mixture of liposome pellets and supernatant. Before being processed further, the pellet containing completely liposomes was resuspended in distilled water. After being completely absorbed in 10 ml of methanol, the drug-free liposomes were sonicated for ten minutes. The drug was released when the vesicles were broken, and the drug content was then calculated. The drug's absorbance was measured at 221.40 nm. The following formula was then used to determine the entrapment efficiency.

Amount of drug Entrapped = Amount of drug present in supernatant – total amount of drug added.

% Entrapment efficiency = (Entrapped drug/Total drug added) X 100

4. Morphology (SEM / TEM)

The electron beam from a scanning electron microscope was used to attain the morphological features of the extract loaded liposome were coated with a thin layer (2-20 nm) of metal(s) such as gold, palladium, or platinum using a sputter coater under vacuum.

5. In vitro drug Release Studies

A modified Franz diffusion cell was used for in vitro release studies. The donor and receptor compartments have been separated by a dialysis membrane (HiMedia molecular weight 5000). The donor compartment was filled with liposomal suspension, while the receptor compartment was filled with 18 ml of pH 7.4 phosphate buffer. Throughout the experiment, the diffusion cells were maintained at 37±0.5°C and stirred at 200 rpm.

PREPARATION OF LIPOSOMAL GEL

Composition of preparation of liposomala gel

S.NO	INGREDIENT	QUANTITY	ROLE
1	Carbopol 934/940	0.5-2%w/w	Gelling agent
2	Centella Asiatica loaded liposomal suspension	0.5-2%w/w	Active ingredient
3	Propylene glycol	5-10% w/w	Penetration enchancer & Humectant
4	Methyl paraben	0.15%w/w	preservative
5	Propyl paraben	0.05%w/w	preservative
6	Triethanolamine	q.s	pH Adjustment
7	Purified water	q.s	vehicle

General procedure

- The appropriate quantities of powdered carbopol 934/940 was introduced into distilled water while being constantly stirred with a glass rod in order to avoid the formulation of indispersible precipitates.
- It was eventually placed to hydrate for a full day at room temperature to cause swelling.
- Dissolve methyl paraben and propyl paraben in warm water and stirred continuously to make stiff gel.
- Add this preservative solution to the hydrated carbopol dispersion.
- Employing a mechanical stirrer (25 rpm, 2 m), liposomes containing gotu kola leaf extract was incorporated into the carbopol gel to produce topical liposome gel formulations.
- Triethanolamine was added to neutralize the PH in the dispersion.
- At this stage permeation enhancer (Propylene Glycol) was added.
- The final dispersion was agitated until smooth gel was formed without lumps.

EVALUATION OF LIPOSOMAL GEL

1. Physical appearance

The prepared gel formulation was evaluated for appearance, colour, odour, and homogeneity by visual observation**.**

2. Measurement of pH

The pH of various gel formulations was determined by using digital pH meter. One gram of gel was dissolved in 100 ml distilled water and stored for two hours. The measurement of pH of each formulation was done in triplicate and average values are calculated. If slight deviations in pH were noted, it was adjusted to skin pH using drop wise addition of tri-

ethanolamine solution.

3. Viscosity study

The measurement of viscosity of the prepared gel was done with a Brookfield Viscometer. The gels were rotated at 1.5 rotations per minute and viscosity was measured in Cps.

4. Spreadability

An ideal topical gel should possess a sufficient spreading coefficient when applied or rubbed on the skin surface. This was evaluated by placing about 1g of formulation on a glass slide. Anotherglass slide of the same length was placed above that, and a mass of 50 mg was put on the glass slide so that the gel gets sandwiched between the two glass slides and spreads at a certain distance. The time taken for the gel to travel the distance from the place of its position was noted down.Spreadability was determined by the following formula.

$$S = M \times L/T$$

Where,

S-Spreadability, g.cm/s M-Weight put on the upper glass

L-Length of glass slide T-Timefor spreading gel in sec

5. Drug Content and Content Uniformity

The gel sample (100 mg) was withdrawn and drug content was determined using UV spectrophotometer.Similarly, the content uniformity was determined by analyzing drug concentration in gel taken from 3 to 4 different points from the container. In case of liposomal gel, it was shaken with sufficient quantity of methanol to extract the drug and then analysed by using UV spectrophotometer.

6. Stability studies

After being packed, the extract-loaded gel formulation was placed in the stability test chamber and placed through three months of accelerated testing at 250C±20C and 60±5% RH and 400C±20C and 70±5% RH. Viscosity and pH were measured for the formulation at intervals of 30, 45, 60, and 90 days (three months). In compliance with International Conference on Harmonization (ICH) requirements, the formulation was tested for stability under accelerated storage conditions for three months. The formulation was examined for changes in the evaluation parameters of pH and viscosity. The final formulation of 0 days served as the reference for all results.

CONCLUSION

The review on the formulation and evaluation of liposomal gel containing Centella asiatica for wound healing activity highlights the significant potential of this novel drug delivery system in enhancing topical wound therapy. Centella asiatica, rich in bioactive constituents such as asiaticoside, madecassoside, and asiatic acid, is well known for its wound healing, anti-inflammatory, antioxidant, and collagen-stimulating properties. Incorporation of Centella asiatica into liposomal carriers offers several advantages, including improved drug stability, enhanced skin penetration, controlled drug release, and reduced degradation of active phytoconstituents. Conversion of liposomal suspension into a gel dosage form further improves patient compliance, ease of application, prolonged residence time at the wound site, and localized therapeutic action. Evaluation parameters such as particle size, zeta potential, entrapment efficiency, pH, viscosity, spreadability, in-vitro drug release, and stability studies collectively demonstrate that liposomal gel formulations can be optimized to achieve effective and consistent performance. Reported wound healing studies indicate accelerated wound contraction, improved epithelialization, and enhanced collagen deposition when compared to conventional formulations. Overall, this review concludes that liposomal gel of Centella asiatica represents a promising and effective topical delivery system for wound healing applications. The formulation combines the therapeutic benefits of herbal medicine with the advantages of nanotechnology, offering a safer and more efficient alternative to conventional wound care therapies. Further preclinical and clinical investigations are recommended to establish long-term safety, efficacy, and commercial feasibility of this delivery system.

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