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Novel Vesicular Formulation Based on A Herbal Extract Loaded With Niosomes and Evaluation of Its Anti-Fungal Potential

Kirti Goel[1*], Rakesh Kumar[2], Poonam Gangwar[3], Victor Roychowdhury[4], Tilottama Bhattacharya[5], Jyoti Thakur[6]

[1*]Maharishi Markandeshwar, College of Pharmacy, Maharishi Markandeshwar Deemed To Be University, Mullana, Ambala, India
[2]B M College of Pharmacy, Farukhnagar, Gurugram, Haryana, India
[3]School of Pharmaceutical Sciences, Shri Guru Ram Rai University, Patel Nagar, Dehradun, Uttarakhand, India
[4]JIS University, Deshpriya nagar, Agarpara, West Bengal, India
[5]Sister Nivedita University, Chakpachuria, West Bengal, India
[6]NRI Institute of Research & Technology-Pharmacy (NIRTP), Bhopal, India

Corresponding Author: [1*]Kirti Goel

Abstract: Current scientific technologies are merged with herbal drug-loaded delivery systems to improve the therapeutic value of pharmaceuticals. This study aims to develop and assess a niosomal drug delivery system based on hydro-alcoholic root extract of *Leonotis nepetaefolia* (L.) R.Br. Particle size entrapment efficiency and in-vitro drug release studies were used to analyse the niosomal dispersion. The use of niosomal formulations for drug administration is becoming more and more popular these days, and *Leonotis nepetaefolia* (L.) R.Br. hydro-alcoholic root extract may find a home in this method for treating fungal infections. The most promising formulation, according to the data, was HNF-8, which included Tween 40 as a surfactant in a 2:1 ratio with cholesterol.

Keywords: *Leonotis nepetaefolia* (L.) R.Br., Niosomes, Fungal infection

1. Introduction

The most common cause of infection worldwide is now fungus-related infections. Human pathogenic organism resistance has been widely documented from around the globe in recent years. However, the widespread use of antibiotics is concerning in both wealthy and poor nations. The resistance of bacterial and fungal infections has further hampered the management of infectious disorders in patients with impaired immune systems. Nowadays, the majority of diseases are caused by common fungal infections including blastomycosis (caused by Blastomyces), aspergillosis (caused by Aspergillus), and candidiasis (caused by yeast-like fungus Candida albicans). These infections are also more likely to affect humans. These species develop quickly in temperatures between 25 and 37^0 C. Depending on the type of underlying host deficiency, these fungal infections can cause a range of infections by colonising the mucosal surfaces of the digestive system, oral cavity, and vagina. Important risk factors for fungal infections include a weakened or immature immune system, metabolic diseases including diabetes, HIV/AIDS, stress, dietary deficiencies, and mononucleosis. [1]
Topical formulations are designed to kill fungi or the causing organism by efficiently permeating the medications into the stratum corneum, which treats local infections on the epidermis. Benefits of topical formulations include possible self-medication, enhanced patient compliance, localised or focused therapy, and limited systemic absorption of the medicine, which lowers systemic side effects. The drawbacks of topical preparations include skin irritation, allergic reactions, poor penetration into the stratum corneum, poor dermal bioavailability, inconsistent drug levels at the site of infection, greasiness or stickiness of ointments and creams, and uncontrollably evaporating drug from the preparation. [2] Consequently, in order to solve the issues with the current formulations, new topical formulations are required. In an effort to enhance topical formulations, formulation scientists have recently investigated drug delivery methods based on nanoparticles. In order to do this, active medications are precisely delivered to the infection site, improving skin penetration, lowering irritation, and lengthening the duration of action. Antifungal drugs have been encapsulated in a number of

innovative drug delivery methods to increase their effectiveness. Lipid nanoparticles, solid lipid nanoparticles, liposomes, ethosomes, polymeric nanoparticles, microemulsions, and nanoemulsions are a few of them. With the aforementioned information in mind, the current study was conducted to create a safe and effective antifungal formulation utilising a unique technique intended to treat fungal infections.

2. Materials and Methods

Plant Material Collection

In October of 2022, the root was purchased from online source. A botanist verified that the plant sample was *Leonotis nepetaefolia* (L.) R.Br.; voucher specimen No. J/Bot/LNF-037 was assigned, and it is a member of the Lamiaceae family.

Extraction of *Leonotis nepetaefolia* (L.) R.Br.

The root of Leonotis nepetaefolia was dried and ground into a powder to create the raw medicinal ingredient. Methanol was used as the solvent in a heated continuous mode to complete the extraction process. After gathering the methanol-soluble components in the receiver, the residual dissolvent was completely removed using a rotating vacuum evaporator. After being transferred to a light-resistant container, the completed product was hermetically sealed. [3]

Preparation of niosomes

Selection of surfactants for niosomes formation

For this investigation, various nonionic surfactants with grades of span 20, span 60, tween 40, and tween 60 were chosen.

Preparation of Drug Loaded Niosome

Thin film hydration process was used to manufacture HAELN (Hydroalcoholic extract of Leonotis nepetaefolia) loaded niosomes utilising several nonionic surfactants (span 20, span 60, tween 40 & tween 60) grades in varied drugratios of surfactant to cholesterol are 1:1:1, 1:2:1, and 1:1:2. Using a 100 ml round-bottom flask, precisely weighted amounts of surfactant and cholesterol were dissolved in 5 ml of chloroform. A rotary shaker was used to evaporate the lipid solution. Until a dry and smooth lipid coating was formed, the flask was spun at 135 revolutions per minute. For three hours, the film was gently shaken while being hydrated with 5 millilitres of pH 7.4 phosphate buffer saline (PBS) containing medication. For a whole day, the niosomal suspension was maintained at 2–8ºC to further stabilise it.[4-5]

Preparation of Niosomal gel

Formulation of 2.5% HAEELN gel

Using carbopol-934 as a gelling agent, gel was created. The necessary amount of gelling agent was weighed and mixed with an adequate amount of distilled water. Triethanolamine was added dropwise to this dispersion until a clear gel was achieved. After dissolving MELN in propylene glycol, a 2.5% w/w gel was produced, and it was processed as previously described. [6-7]

Incorporation of niosomes of MELN to gel base

The 2.5% w/w HAELN niosomal formula equivalent was added to the gel basis by gently mechanically mixing it for 15 minutes at 25 rpm.

Table 1: Composition of Niosomal of *Leonotis nepetaefolia* root extract

Formulation Code	Surfactant used	Drug (MELN): Surfactant:Cholesterol Ratio	Solvent	Weight taken (mg)
HNF-1	Span 20	1:1:1	Chloroform	100:100:100
HNF-2		1:2:1	Chloroform	100:200:100
HNF-3		1:1:2	Chloroform	100:100:200
HNF-4	Span 60	1:1:1	Chloroform	100:100:100
HNF-5		1:2:1	Chloroform	100:200:100
HNF-6		1:1:2	Chloroform	100:100:200
HNF-7	Tween 40	1:1:1	Chloroform	100:100:100
HNF-8		1:2:1	Chloroform	100:200:100
HNF-9		1:1:2	Chloroform	100:100:200
HNF-10	Tween 60	1:1:1	Chloroform	100:100:100
HNF-11		1:2:1	Chloroform	100:200:100
HNF-12		1:1:2	Chloroform	100:100:200

Table 2: Formulation of 2.5 % HAELN gel

Ingredients	**Quantity (100 gm)**
HAELN (2.5%)	2.5 gm
Carbopol 934 (2%)	2 gm
Propylene glycol (10%	9.6 ml
Triethanol amine	qs
Distilled water	qs

Characterization of niosomes
Niosomes formulations were characterized using standard procedures. [4-5]
Vesicle shape and morphology
Shape and morphology of niosomal formulations were determined by optical microscopy and Scanning Electron Microscopy (SEM).
Particle size
The particle size of the niosomal suspension was determined by optical microscopy. A drop of niosomal suspension was placed on a glass slide. A cover slip was placed over the niosomes suspension and evaluated the average vesicle size by an ordinary optical microscope using a pre calibrated ocular eye piece micrometer.
Entrapment Efficiency
Entrapment efficiency of niosomal formulations was determined by centrifugation method. 10mL niosomal suspension was poured into a stopper test tube and centrifuged by using cooling centrifuge at 10,000 rpm maintained at 4^0Cfor 90minutes and then filtered by using Whatman filter paper to obtain clear fraction. The clear fraction was used for the determination of free drug by using UV spectrophotometer at 335 nm respectively. The encapsulation efficiency was calculated using the formula

EE (%) = [(Ct-Cf)/Ct] ×100
Where, Ct is concentration of total drug; Cf is concentration of unentrapped drug.
Drug Content
Drug content was determined by disrupting the niosomal formulation by propane-1-ol, diluted suitably using phosphate buffer pH 6.8 and analysed for the drug content spectrophotometrically at 335 nm respectively.
Evaluation of niosomal gel and plain gel
The niosomal gel and plain topical gel were characterized with respect to pH, viscosity, and spreadability. [6-7]
Physical examination
The plain gel and niosomal gel was visually examined for color and texture.
pH Measurements
The pH of the gel formulations was delivered by using digital pH meter.
Viscosity Measurement
The viscosity of gel formulations was determined by Brookfield viscometer. 25.0 g gel was taken in beaker and spindle number 4 was rotated at 50 rpm and viscosity of the sample was determined.
Spreadability
The spreadability of gel formulations was determined by using spreadability apparatus. 1.0 g of gel sample was placed on the lower slide and upper slide was placed on the top of the sample.The spreadability was determined by the formula
S=m X l/t
Where, S is spreadability, m is weight tied to upper slide, l is length travel by upper slide and t is time.
Antifungal Activity of formulation [8]
The antifungal activity of plain and niosomal gel was performed by disc diffusion method against *Aspergillus flavus* and *Candida albicans*. ZOI was recorded and presented in results.

3. Results and Discussion

The in vitro drug release profile and the particle size, shape, and entrapment efficiency of developed niosomal formulations were characterised. It was evident that all of the manufactured batches' niosomes have a spherical form. The niosomal formulation's mean particle size was determined to be between 1.49 and 5.82 μm. Figure made it very evident that adding more cholesterol increased the particle size of niosomal formulations. When the cholesterol ratio was raised from t to 2, the niosomal formulation's entrapment efficiency rose. According to the

thin film hydration method, the drug content ranged from 96.29% to 98.18%. The amount of surfactant in niosomal formulations was found to have an impact on the drug content. The drug content rose in tandem with the amount of surfactant in the niosomal formulation. This phenomenon may be explained by a reduction in drug leakage, which enhances both the drug content and entrapment efficiency.

Table 3: Evaluation Parameters of Niosomal formulation containing root extract of LN

Formulation Code	Particle size (µm)	EE(%)	Drug content (%)
HNF-1	5.82±1.10	64.20±0.05	96.29±0.23
HNF-2	4.82±1.11	71.22±0.11	97.12±0.01
HNF-3	5.96±0.32	71.12±0.26	97.12±0.11
HNF-4	2.17±0.28	68.11±0.29	96.69±0.32
HNF-5	3.12±1.28	71.24±0.30	97.72±0.22
HNF-6	4.33±1.23	79.38±0.24	97.59±0.05
HNF-7	1.49±0.13	81.31±0.39	97.89±0.20
HNF-8	2.78±1.11	83.24±0.31	98.18±0.17
HNF-9	3.92±0.12	79.11±0.31	97.51±0.19
HNF-10	1.02±1.29	70.01±0.29	96.10±0.32
HNF-11	2.11±0.24	71.19±0.22	96.19±0.24
HNF-12	2.17±0.29	76.37±0.06	96.19±0.11

All reading are expressed as mean ± S.D. (n = 3)

The formulation code HNF-8 was found to have the highest entrapment efficiency and drug content based on results from in vitro diffusion studies and entrapment efficiency and drug content studies of niosomal formulation. Consequently, these three formulations were taken and incorporated to form niosomal gel. Additionally, a plain gel was made using the techniques outlined in the approach. Every prepared formulation was assessed. The outcomes were displayed below.

Table 4: Physical examination and other characterization of plain and niosomal gel formulation containing MELN

Formulation Code	Color	Homogenity	Texture	pH	Viscosity (cps)	Spreadability (gmcmsec)	Drug Content
PGHAELN	White	Homogeneous	Smooth	7.11	7012	18.12	97.33±0.07
HNF-8	White	Homogeneous	Smooth	6.98	6814	18.89	98.36±0.11

4. Conclusion

The statistics clearly show that HNF-8, which included Tween 40 as a surfactant in a 2:1 ratio with cholesterol, was the most promising formulation.

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