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Formulation and Evaluation of Niosomes of *Leonotis nepetaefolia* (L.) R.Br.

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Abstract

Herbal drug loaded delivery strategies are combined with current scientific technologies, which enhance the therapeutic value of the pharmaceuticals. The objective of this research is to formulate and evaluate *Leonotis nepetaefolia* (L.) R.Br. hydroalcoholic extract-based niosomal drug delivery system. Analysis of the niosomal dispersion was conducted using particle size entrapment efficiency and in-vitro drug release study. Drug delivery through niosomal formulations is gaining success rapidly at the present time and it could be a suitable carrier for *Leonotis nepetaefolia* (L.) R.Br. hydroalcoholic extract for the treatment of fungal infection. The results indicates that most promising formulation was NFLN8, which contained Tween 40 as surfactant at ratio of 2:1 with cholesterol.

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

Introduction

Fungal infections have become world's leading cause of infection. In recent years, resistance to human pathogenic organisms has been frequently reported from all over the world. However situation is alarming in both developing as well as developed countries due to indiscriminate use of antibiotics. The treatment of infectious diseases in immune compromised patients has become further complicated due to the resistance of bacterial and fungal pathogens. Most common fungal infections such as candidiasis (caused by yeast like fungus *Candida albicans*), aspergillosis (caused by Aspergillus), blastomycosis (caused by Blastomyces) etc. are now a day's more prone to human and causing majority of diseases. These species grow rapidly at 25- 37^0 C temperature. These fungal infections colonize mucosal surfaces of the oral and vaginal cavities and the digestive tract and are able to cause variety of infections depending upon the nature of the underlying host defect. Weak or immature immune system or metabolic illness such as diabetes, HIV/AIDS, stress, nutrient deficiency, mononucleosis is important predisposing factors for fungal infections. [1]

Topical formulations are intended to treat local infections on the topmost layer of the skin by effectively penetrating the drugs into the stratum corneum, thus destroying the fungi or the causative organism. Advantages associated with topical formulations include limited systemic bioavailability of the drug, which reduces the systemic adverse effects, potential self-medication, increased patient compliance, and targeted or localized therapy. However, topical preparations have disadvantages such as poor dermal bioavailability, poor penetration into the stratum corneum, variable drug levels at the site of infection, greasiness or stickiness of ointments and creams, skin irritation, allergic reactions, and uncontrolled evaporation of drugs from the preparation. [2]

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Therefore, there is a need for novel topical formulations to address the problems associated with the current existing formulations. Recently, formulation scientists have explored nanoparticle-based drug delivery systems to improve topical formulations. This is done by delivering active drugs precisely to the infection site while enhancing skin penetration, reducing irritation, and increasing the sustained effect. Several novel drug delivery systems have been formulated to encapsulate antifungal agents and improve their efficacy. Some of them include microemulsions, nanoemulsions, niosomes, dendrimers, solid lipid nanoparticles, liposomes, ethosomes, lipid nanoparticles, and polymeric nanoparticles. Keeping the above facts in view the present work was undertaken to develop an effective and safe antifungal formulation using novel approach indented for the treatment of fungal infection.

Materials and Methods

Plant Material Collection

The plant part (Root) was collected from the Malwa region of Madhya Pradesh in the month of August-2020. The plant sample was authenticated as *Leonotis nepetaefolia* (L.) R.Br. by Botanist and Voucher specimen No. J/Bot/LNR-11 was assigned and it belongs to family Lamiaceae.

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

The crude drug material was prepared by dehydrating and pulverising *Leonotis nepetaefolia* root. The extraction process was accomplished in a hot continuous mode utilizing methanol as the solvent. The rotary vacuum evaporator was used for absolute remotion of leftover dissolvent after collecting the methanol dissoluble components in the receiver. The finished product was moved to a light-resistant container and hermetically sealed. [3]

Preparation of niosomes

Selection of surfactants for niosomes formation

The different nonionic surfactants viz., span 20 was selected for present study.

Preparation of Drug Loaded Niosome

HAELN (Hydroalcoholic extract of *Leonotis nepetaefolia*) loaded niosomes were formulated by using thin film hydration technique and the different nonionic surfactants (span 20) grades in different drug:surfactant:Cholesterol ratios as 1:1:1, 1:2:1, 1:1:2. Accurately weighted quantities of surfactant and Cholesterol were dissolved in 5 ml chloroform using a 100 ml round bottom flask. The lipid solution was evaporated by rotary shaker. The flask was rotated at 135 rpm until a smooth and dry lipid film was obtained. The film was hydrated with 5 ml phosphate buffer saline (PBS) of pH 7.4 containing drug for 3 hours with gentle shaking. The niosomal suspension was further stabilized by keeping at 2-8ºC for 24 hours. [4-5]

Preparation of Niosomal gel

Formulation of 2.5% HAELN gel

Gel was prepared using carbopol-934 as gelling agent. Required quantity of gelling agent was weighed and dispersed in sufficient quantity of distilled water. This dispersion was neutralized by drop wise addition of triethanolamine till a clear gel was obtained. A 2.5% w/w gel was obtained by dissolving HAELN in propylene glycol, and treated in the same way as explained above. [6-7]

Incorporation of niosomes of HAELN to gel base

Selected niosomal formula equivalent of 2.5% w/w HAELN was incorporated into gel base by gentle mechanical mixing at 25 rpm for 15 min.

Table 1: Composition of Niosomal of *Leonotis nepetaefolia*

Formulation Code	Surfactant used	Drug (HAELN): Surfactant:Cholesterol Ratio	Solvent	Weight taken (mg)
F1	Span 20	1:1:1	Chloroform	100:100:100
F2		1:2:1	Chloroform	100:200:100
F3		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

$$EE\ (\%) = [(Ct\text{-}Cf)/Ct] \times 100$$

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

$$S = m \times l/t$$

Where, S is spreadability, m is weight tied to upper slide, l is length travel by upper slide and t is time.

Results and Discussion

Developed niosomal formulations were characterized with respect to particle size, shape, entrapment efficiency, and *in vitro* drug release profile. It was clearly observed that niosomes are spherical in shape for all prepared batches. The results were given in table 3 and for niosomal gel the results were presented in table 4.

Table 3: Evaluation Parameters of Niosomal formulation containing HAELN

Formulation Code	Particle size (µm)	EE(%)	Drug content (%)
F1	5.77±1.11	68.20±0.12	96.22±0.04
F2	4.81±1.02	71.23±0.12	97.39±0.15
F3	5.90±0.43	73.01±0.02	97.48±0.11

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

On the basis of results obtained of entrapment efficient and drug content and *in vitro* diffusion studies of niosomal formulation it was found that the formulation code F3, was found to have highest entrapment efficiency and drug content, therefore these three formulation were taken and was incorporated to form niosomal gel. A plain gel was also prepared as per methods described in methodology. All the prepared formulations were evaluated. The results were presented below.

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

Formulation Code	Color	Homogenity	Texture	pH	Viscosity (cps)	Spreadability (gmcmsec)	Drug Content
PGHAELN	White	Homogeneous	Smooth	7.0	6245	18.49	95.12±0.04
F3NG	White	Homogeneous	Smooth	7.1	6122	15.29	97.33±0.01

Conclusion

From the results obtained it was depicted that F3 NG promising formulation containing Span 20 as surfactant at ratio of 1:1:2 with cholesterol and is novel formulation results were excellent than plain gel as compared and presented in the results.

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